STUDY OF COMPLEMENT IN CHILDREN WITH CEREBRAL MALARIA

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C E R T I F I C A T E

This is to certify that the work entitled "STUDY OF COMPLEMENT IN CHILDREN WITH CEREBRAL MALARIA" has been carried out by MOHAMMAD PARVEZ MAJID under my direct supervision and guidance in the Department of Paediatrics, M.L.B. Medical College, Jhansi. Techniques employed and observations made were conducted by the candidate himself.

He has put in the necessary stay in the department according to University regulations.

Dated: 10.9., 1988.

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Dated: 6.9., 1988.

(MOHAMMAD PARVEZ MAJID)

C O N T E N T S

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INTRODUCTION

INTRODUCTION

Malaria is known to humanity since the dawn of civilization. Even though it has a world wide distribution, yet it is the main cause of morbidity and mortality in tropics only. Among its complications cerebral malaria has got the distinction of having the highest mortality rate. For a clinician the term cerebral malaria encompasses the development of even a mild cerebral dysfunction during the course of malarial infection. But for the sake of discerning researcher an expert committee instituted by the W.H.O. Malaria Action Programme (1986) recommended that a strict definition given by Warrell et al in 1982 be followed. The definition requires :

1. presence of unarousable coma i.e. the motor response to noxious stimuli is nom localizing or absent.
2. exclusion of other encephalopathies. The coma should persist for more than six hours after a generalised convulsion to exclude transient post ictal coma. Hypoglycemia, meninogencephalitis, eclampsia, intoxication, head injury, cerebrovascular accident and/or a metabolic disorder should be excluded as being the cause of coma.
3. Confirmation of Plasmodium falciparum infection. Asexual froms of Plasmodium falciparum must be demonstrated in the peripheral blood or bone marrow smear (during life) or in brain smear (after death).

The aetiology of cerebral malaria is controversial. Though there are several reports in the literature implicating Plasmodium vivax, yet many authors claim that apart from plasmodium falciparum no other species of malarial parasite causes cerebral malaria in human beings. Regarding the reported cases of cerebral malaria as being due to Plasmodium vivax it has been proposed that either some neuroviral infection was associated or mixed infection due to Plasmodium falciparum was overlooked in the presence of more uniformly distributed Plasmodium vivax infection.

The pathogenesis of cerebral malaria has been a challenge to research workers. From time to time various theories have been proposed only to be discarded later. But the essential pathological feature of cerebral malaria is the great sequestration of erythrocytes containing mature forms of the parasite, nemely, Plasmodium falciparum, in the deep vascular bed of the brain.

Among the various theories of pathogenesis of cerebral malaria the permeability theory which puts the increased capillary permeability of cerebral vasculature as the reason for development of cerebral oedema and subsequently coma has got certain discrepancies. This theory which is based on animal studies, is not consistent with the findings in human cerebral malaria.

The mechanical theory states that sequestration of parasitized erythrocytes occurs due to decreased deformability of such cells as compared to normal erythrocytes. Again, this theory fails to explain why obstruction does not occur at the narrowest points in the brain vasculature and why Plasmodium vivax which has got larger size, rendering the parasitized erythrocytes more rigid, is a less frequent cause of cerebral malaria.

Perhaps the discovery of 'Knob' like protrusions on parasitized RBC's and presumed preponderance of corresponding receptors in cerebral vasculature has given the greatest impetus to workers favouring mechanical theory. This may explain the capillary blockage in brain due to sticking of such cells to their receptors present on endothelial lining.

However, immunologists are working the other way round. Hypocomplementemia in malaria and its complications is well documented. It has been linked to the prognosis of the patient i.e. lower the level of complement poorer the prognosis. It has been assumed that complement activation by parasitized red blood cells or circulating immune complexes formed by malarial antigens and antibodies are responsible for damage to the cerebral vasculature.

Yet another evidence in favour of immunological mechanism is the experimental demonstration of deposition of immune complexes in choroid plexus in murine malaria.

In fact, Toro and Roman (1978) went on to propose the term 'hypergic' immune complex vasculitis to explain certain characteristics of cerebral malaria which are reminiscent of immune complex disease. Adams et al (1981) found evidence of circulating immune complexes in cases of cerebral malaria associated with hypocomplementaemia. The level of circulating immune complexes increased after quinine therapy possibly due to release of malarial antigens leading to deepening of coma.

The present study was undertaken to evaluate the aetiological organism in cerebral malaria in paediatric age groups to assess the levels of complement components, C3 and C4, in the serum and demonstrate their linkage to circulating immune complexes. Mortality rate of cerebral malaria is high in children (about 25 percent). The diagnosis of cerebral malaria poses a greater enigma and therefore a high index of suspicion has to be maintained. Data regarding the level of complement components linked to immune complexes is scanty. There is a great paucity of complement studies, in general, in the Indian literature. For these reasons a need was felt to conduct a study while selecting the sample according to the strict definition given by Warrell et al (1982). However, almost all the previous studies included even those cases of malaria who had the mildest cerebral dysfunction.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

MALARIA

It is an acute infectious disease caused by plasmodia transmitted by mosquito of genus anopheles. This illness is characterized by a cyclic course with periods of acute febile attacks and paroxysm free intervals as well as by hepatosplenomegaly, anaemia and occasional severe lesions of nervous system, kidneys and other organs (Loban and Polozak, 1985).

CEREBRAL MALARIA :

Cerebral malaria is a dreaded complication of malaria especially in paediatric age group, carrying a high fatality rate of about 25%, despite best possible therapeutic measures (Bruce Chwatt, 1978). It is characterized by gradual or sudden development of repeated convulsions somnolence, stupor and finally coma (Bruce Chwatt, 1978).

HISTORICAL BACK-GROUND

According to Loban and Polozak (1985) malaria has been known to humanity since the dawn of civilization. References to epidemic fever, similar to malaria can be found in ancient Chinese and Egyptian manuscripts and also the literary sources of ancient greece and Rome. According to Bergin (1967) from 460-370 B.C. Hippocrates, in his book on epidemics noted the existance of periodic fever divided into quotidian, tertian, quartan and subtertian, which was associated with enlarged spleen.

1638 AD - Hu an del Vega first employed cinchona bark for the treatment of malaria.

1753 AD - Term malaria was coined.

1820 AD - Active principle of cinchona bark (quinine) was isolated.

1931 AD - Bright noted the pigmented appearance of spleen and brain at autopsy.

1880 AD - Laveran discovered the malarial parasite in an unstained preparation of fresh blood.

1881 AD - Laveran described plasmodium malariae (P. malariae).

1883 AD - Marchiafava used meltylene blue for staining of malaria parasite.

1885 AD - Golgi demonstrated erythrocytic schizogony of quartan malarial parasite.

1886 AD - Golgi demonstrated the erythrocytic schizogony of benign tertian malarial parasite.

1890 AD - Grassi and Feletta described plasmodium vivax (P. vivax) and P. malariae.

1891 AD - Romanonowsky introduced the staining method of malarial parasite.

1897 AD - Welch identified plasmodium falciparum (P. falciparum). Ronald ross in secundrabad found oocysts on the stomach wall of anopheline mosquito which had previously fed on a malarial patient.

1898 AD - Ross worked out mosquito cycle of avian malaria while Bignami worked out that of human malaria.

1900 AD - Manson confirmed the mosquito transmission of malaria.

1922 AD - Stephens in Africa discovered plasmodium ovale (P. ovale).

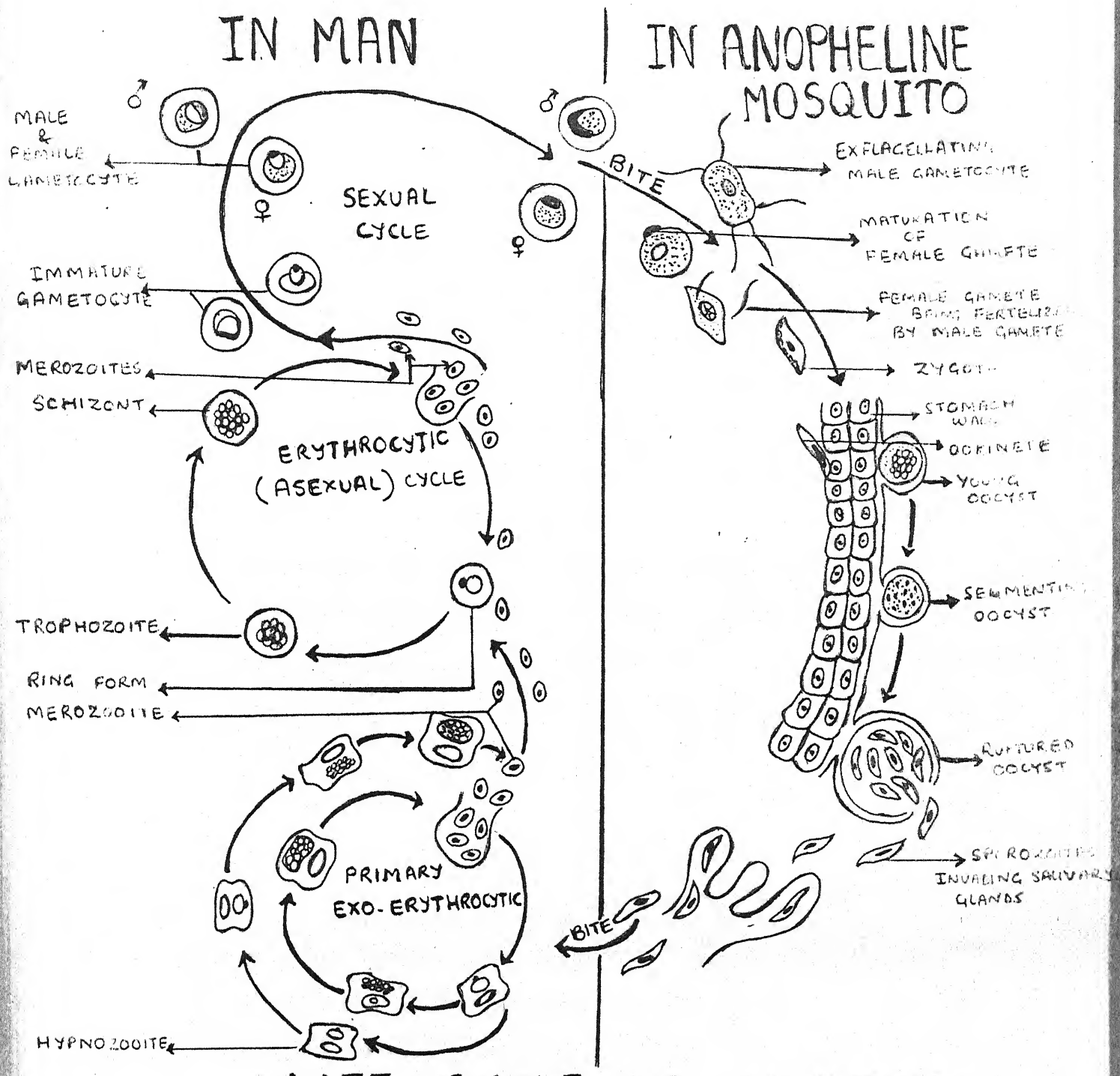
1948-49 AD - Short et al identified tissue forms of P. vivax and P. falciparem.

1960 AD - Emergence of a drug resistant strain of P.falciparum was noted in South America, Africa and South East Asia.

Aetiology of Malaria :

Malaria occurs following the bite of Anopheles mosquito which transmits causative agents of malaria to human host. Causative agent a protozoa, belongs to class sporozoa order Haemosporidia, family Plasmodidae and genus plasmodium.

Man may host four types of plasmodia namely P. vivax, P. falciparum, P. malaria and P. ovale. He is also susceptible to several types of malarial parasites in monkeys. P. Knowlesi, P. cynomolgi, P. cynomongi bastianelli, P. inui, P. brasilianum, P. shortie, P. simium which may be contracted not only in experimental conditions but also under natural conditions with the subsequent transmission of infection to another man via mosquitoes (Demina 1968, Fong, cadigan, 1971).



LIFE CYCLE OF MALARIAL PARASITE

(AFTER CRUICKSHANK-1973)

FIG-1

et al, 1978). For P. vivax, such receports are presented by isoantigens of blood group Daffy (Fy^A/Fy^B) and glycophorines of erythrocytic membrane appear to serve as receptors for P. falciparum.

Following their penetration into erythrocytes merozoites enlarge to form trophozoites and undergo various stages of development-ring trophozoite, juvenile trophozoite, adult trophozoite, immature and then mature schizont which consists of 8-24 merozoites depending on the type of parasite. After the completion of the developmental cycle the erythrocyte is destroyed and merozoites become blood borne. Some of them perish, others attack fresh red blood cells within 10-15 minutes and invade them. Each cycle of erythrocytic schizogony lasts 48 to 72 hours. In P. vivax, ovale and falciparum it is 48 hours and 72 hours in P. malariae.

(c) Gametogony (Leban and Polonsk, 1985) :

Some of the merozoites in the red blood cells generate sexual forms of the parasite (male and female gametocytes). Mature gametocytes of P. vivax, P. ovale, and P. malariae appear in the peripheral blood almost simultaneously with asexual forms and are detected during first attack of the disease. Gametocytes of P. falciparum get mature within 10-12 days and appear in the peripheral blood only 8-10 days after the onset of the disease.

(d) Exo-erythrocytic schizogony (Garnham 1966) :

Until recently it has been considered that in the recurrent forms of human malaria (P. vivax and P. ovale infections) not only pre-erythrocytic but also exo-erythrocytic schizogony takes place as a result of repeated penetration by merozoites (formed in the course of pre-erythrocytic schizogony) into the hepatic cells of tissue. But this hypothesis has now been challanged and there is more evidence of latent tissue stage (hypnozoites) in the hepatic cells for P. vivax and P. ovale. As regards P. malariae some recent evidence indicates that their relapses may originate from erythrocytic forms remaining in the body for a considerable period of time.

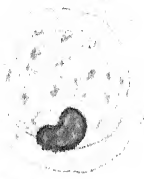
2. Mosquito cycle (Sporogony) - (Loban & Polosak, 1925) :

Mosquitoes of genus Anopheles are infected from a malaria patient or a malaria carrier. Along with the blood meal sexual forms of Plasmodia get into the stomach of mosquito wherein a macrogamete is formed from the female gametocyte. The male gametocyte extrudes 4 to 8 flagellate microgametes which later break free and enter into the female sexual cycle, fertilising it. The fertilized ovum, zygote, is transformed into the ookinete which penetrates through the wall of mosquito stomach reaching external membrane where the ookinete gets round and turns into the oocyst surrounded by a membrane. The oocyst grows, its content and multiplies resulting in

PLASMODIUM VIVAX

TROPHOZOITE

SCHIZONT



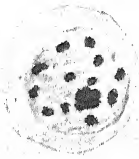
FEMALE

MALE

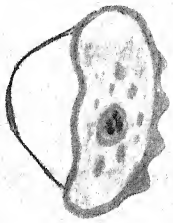
GAMETOCYTES

PLASMODIUM FALCIPARUM

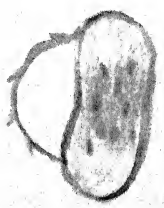
TROPHOZOITE



SCHIZONT



FEMALE



MALE

GAMETOCYTES

(AFTER WHO 1983)

formation of large amount of sporozoites (upto 10,000) of spindle like form.

After the maturation, the oocyst membrane ruptures, sporozoites are released and together with haemolymph are spread throughout the organism, accumulating in large quantities in salivary glands of mosquito. Such a mosquito is infectious for man remaining in this state for 1-2 months.

CLINICAL FEATURES OF MALARIA (BRUCE CHWATT, L.J. 1978) W.H.O. 1986) :

After initial infection there follows an incubation period of 12 days (9-14) days for falciparun malaria, 14 (8-17) days for vivax malaria, 28 (18-40) days for quartan malaria and 17 (16-18) days for ovale malaria. This duration may be prolonged with some strains of P. vivax and due to prophylaxis which is inadequate to destroy completely the developing parasite (W.H.O. 86).

In adults, who have little immunity to the disease, a classical febrile paroxysm of the primary attack is preceded by premonitary signs (headache, anorexia, lassitude, nausea) and is composed of a cold stage with feeling of chill accompanied by a rigor, a hot stage with dry flushed skin, rapid respiration and thirst. This is followed by a sweating stage when the temperature falls by crisis, the patient perspires copiously and feels greatly relieved but very weak.

After a brief period of remittent fever at the begining of the disease, all types of malaria tend to show a periodicity which is more pronounced in vivax ovale infections (tertian, with attack every second day) and malariae infection (quartan, with attack every third day). It is least obvious in falciparum infection.

In children the classical features of malarial paroxysm, as seen typically in non immune adults, are not common.

In non immune infants and children who contract an acute primary attack some variability often occurs. The child at first appears listless, restless or drowsy, refuses food and may complain of headache and nausea. Pallor and cyanosis may be seen. Thirst may be marked, especially after rise of temperature which in breast fed infants displays as repeated attempts to suck, but soon this is abandoned possibly due to nausea. A clear cut cold stage and a definite rigor are uncommon in infants and children, vomiting is often marked, and the vomitus tinged with bile, causing difficulty in feeding, but usually not severe enough to cause dehydration and electrolyte imbalance. Stools are often loose and dark green; mucus may be seen but blood or leucocytes are rare. Infants may appear in abdominal distress. Older children may refer pain to liver or spleen and they may be constipated.

The temperature is very variable, in some is only moderate but in the majority it is high (40°C), often continuous and irregular as the child is flushed and perspires freely. Even when the temperature is moderately high convulsions often occur. They usually last only a few minutes and reflact some cerebral irritation. Hepatosplenomegaly is often found. Splenomegaly develops earlier in vivax malaria, less rapidly in falciparum malaria and very slowly in quartan malaria.

Manifestations are usually masked in immune children. There may be slight restlessness, lack of appetite, sweating anaemia and occassional rise of temperature. However, unsuspected illness may occassionally flare up into a severe complication.

COMPLICATIONS OF MALARIA

A. Falciparum Malaria :

There are a number of complications of Falciparum malaria. First and foremost is cerebral malaria (described later). Other complication include :

(1) Algid Malaria (Collapse) :

The patient may suddenly collapse possibly when the temperature is subnormal. The blood pressure will be low and the pulse weak, often, there has been much vomiting and possibly diarrhoea. Peripheral circulatory failure due in part to dehydration and in part in some cases to lesions in adrenal glands is thought to be responsible for

this complication, which was formerly referred to as the algid type of infection (W.H.O., 1986).

An expert committee of W.H.O. Malaria Action Programme (1986) is of the view that this disease may result from gram negative septicemia based on the studies of Bygbjerg and Lamng (1982).

(2) Severe anaemia :

It is defined as a haematocrit less than 20% (Haemoglobin less than 7.1 g/dl) by an expert committee of W.H.O. Malaria Action Programme (1986).

Approximately 30% of patients require blood transfusion (Phillips et al 1986).

(3) Acute renal failure :

The shock like mechanism associated with severe malaria, particularly when there are cerebral features, may lead to oliguria or anuria and histologically in such cases acute tubular necrosis will be present (W.H.O., 1986). The clinical pattern is that of reversible dysfunction which in a minority of cases progresses to established acute tubular necrosis (W.H.O. Malaria Action Programme, 1986).

(4) Gastrointestinal involvement :

This manifests itself by severe vomiting and diarrhoea, the former may be a prominent symptom in young children, while the latter may be absent, dehydration and electrolyte imbalance may follow (Bruce Chwatt, 1982).

Sweating may further contribute to dehydration (W.H.O., 1986).

(5) Hyperpyrexia :

Hyperpyrexia is defined as rectal body temperature above 39° C (W.H.O. Malaria Action Programme, 1986) which can occur in certain cases of falciparum malaria.

(6) Liver damage :

Abnormalties in liver function tests are common in malaria but they do not necessarily imply impairment of liver function. Jaundice is common, particularly in severe falciparum, malaria resulting mainly from haemolysis (W.H.O. Malaria Action Programme, 1986).

Hepatic dysfunction in severe malaria is usually mild and has apparently been exaggerated in previous reports (Mc Mohan et al 1954; Patwari et al, 1979). Clinical signs of liver failure are never seen unless there is concomitant viral hepatitis (W.H.O. Malaria Action Programme, 1986).

(7) Pulmonary oedema :

This is a grave and usually fatal manifestation of severe falciparum malaria..The first indication of impending pulmonary oedema is usually an increase in the respiratory rate which precedes the development of other chest signs (W.H.O. Malaria Action Programme, 1986).

(8) Bleeding and clotting disturbances :

Disseminted intravascular coagulation (D.I.C.) has been considered an important pathology in severe falciparum malaria (Devakul et al 1966, Jaroonvesamma, 1972, Srikaichul et al 1975). More recently enthusiasm for the role of D.I.C. has declined (W.H.O. Malaria Action Programme, 1986).

Thrombocytopenia is quite common but in most cases not accompanied by bleeding.

(9) Hypoglycaemia :

Hypoglycaemia can occur in severe falciparum malaria, either without the administration of quinine or with administration of quinine which is the most frequent cause of hypoglycaemia (W.H.O., 1986).

(10) Black water fever (W.H.O., 1986) :

Classical black water fever consists of a sudden massive haemolytic episode in which the patient who has felt unwell for sometime takes a dose of quinine and within an hour or two has an attack of shivering, feels weak and collapses and the urine, which till then had been normal in colour is almost black when next passed. Marked anaemia, rapidly develops and recurrent rigors and an irregular fever follows. There is almost always a history of having taken small doses of quinine (inadequate dose to suppress the existing P. falciparum infection) (W.H.O., 1986).

(11) Complicating and associated infections like aspiration bronchopneumonia, U.T.I., gram negative septicemia.

B. Complications of other forms of Malaria (P. vivax, P. ovale and P. malariae) :

(1) Anaemia :

This may develops in any of these malarial infections particularly after repeated attacks or long continued untreated infections (W.H.O., 1986).

(2) Rupture of spleen :

Malaria is an important cause of spontaneous spleen rupture, world wide (Covell, 1955). Eighty percent mortality has been reported in patients in whom malaria infection was induced for fever therapy (Covell, 1955) for the treatment of neurosphilis. Death occurs from loss of blood (Hamilton and Pikacha, 1982).

Spantaneous rupture is more frequent in vivax malaria than in falciparium malaria (Covell, 1955, Martello et al, 1969).

The rapid hyperplastic enlargement of the malarial spleen is important to the pathogenesis (W.H.O. Malaria Action Programme, 1986).

(3) Hepatic dysfunction :

Hepatomegaly and nonspecific hepatitis with or without jaundice occur in patients with vivax malaria (Ramchandran and Perera, 1976, Martello et al, 1969).

In addition to jaundice and hepatomegaly, patients may have fever, constitutional symptoms, hepatic tenderness, mild abnormalities in liver function and transient bilirubinemia (W.H.O. Malaria Action Programme, 1986).

(4) Thrombocytopenia :

Thrombocytopenia occurs in P. vivax infections (Martello et al 1969; Hill et al 1964). However, dangerously low levels are rarely seen.

(5) Cerebral Malaria :

Cerebral Malaria has been described later.

(6) Nephrotic syndrome (W.H.O., 1986) :

A form of the nephrotic syndrome has been encountered particularly in long standing P. malariae infections. It has also very been reported in children with protracted P. ovale infection. It results from deposition on the glomerular basement membrane of immune complexes developed against malarial parasite. In these cases gross generalized oedema, with proteinuria and severe hypoproteinemia occur. Childrens of either sex and about the age of five years are most likely to be affected. The prognosis is usually favourable.

CEREBRAL MALARIA

Cerebral malaria is a dreaded complication of falciparum malaria (Bruce Chwatt, 1978). Most clinicians regard any manifestation of cerebral dysfunction in a patient with malaria as evidence of cerebral malaria (W.H.O. Malaria Action Programme, 1986).

Manifestations of cerebral malaria include any impairment of consciousness (confusion, delirium, stuper, obtundation, coma), convulsive disorders, focal neurological disturbances or psychoses (W.H.O. Malaria Action Programme, 1986). Since fever alone can cause most of these abnormalities, Warrel et al (1982) have developed a strict definition of cerebral malaria to allow a clear cut distinction from mild or transient cerebral dysfunction not related to underlying pathophysiology of cerebral malaria there must be :

(1) Unarousable coma (motor response to maximum stimuli is non-localizing or absent).

(2) Exclusion of other encephalopathies. Coma should persist for more than six hours after a generalized convulsion to exclude transient post ictal coma. Hypoglycemia, meningoencephalitis, eclampsia, intoxications, head injuries, cerebrovascular accidents and metabolic disorders should be excluded as the cause of coma.

(3) Confirmation of P. falciparum infection. Asexual forms of P. falciparum must be demonstrated in peripheral blood or bone marrow smear during life, or in brain smear after death.

But Osuntokun, 1983 described acute cerebral malaria as one characterised by fever, altered sensorium ranging from confusional states to coma, abnormal behaviour, convulsions (in about 50%) and less commonly focal neurological deficit.

Clinical features of cerebral malaria :

Anderson (1927) described that agitation and confusion could develop in a patient of cerebral malaria as he recovered consciousness and transient paranoid psychosis or delirium sometimes followed the acute illness.

Arieti (1946) described as chronic sequalae or presentation of malaria, neurosasthenic syndromes, paranoid, schizoid and manic depressive psychoses.

Taresev (1946) noted that malarial coma could occassionally develop in a patient even with normal temperature. Daroff et al (1967) classified the clinical features of cerebral malaria in 19 patients, viz. disturbances of consciousness (90%), acute organic mental syndromes (25%), movement disorders (10%), focal neurological disorders (50%) and acute personality disorders (15%). Some patients had more than one symptom. They also observed that acute personality changes recovered without any residual disability. They further noticed that in patients having focal neurological disorders, residual deficit was mild and rare.

Hendrickse et al (1971) observed that during the first year of life there was a positive and direct relationship between convulsions and malaria. They have observed that convulsions could occur even at moderate parasitemia. Moreover convulsions were more common in

patients with relatively better packed cell volume and those who were well nourished as compared to anaemic and malnourished children.

Marsden and Bruce Chwatt (1975) have described permanent sequalae of cerebral malaria like deafness, blindness, hemiplegia, cerebral ataxia and choreiform movements (rare).

In 1976, Illan geseka and Desylva described an acute cerebral syndrome in P. falciparum malaria.

Bruce Chwatt (1978) was of the view that cerebral malaria could develop gradually or suddenly and manifest itself in repeated convulsions, somnolence, delirium, stupor and finally coma. Usually the child had been ailing for a few days before the first convulsion. There were few if any, symptoms in the nervous system; some children had shown slight neck stiffness. The cerebrospinal fluid was normal in most cases, though occassionally it was under a slightly increased pressure and there was an increase of cells (up to 20/comm.) and protein (up to 50 mg/100 ml). Even with the best available treatment, the mortality of cerebral malaria in young children could be as high as 25% and those who survived showed neurological sequelae and mental defects as the author reported.

Vietze (1978) has discussed a variety of neurological syndromes associated with malaria. According to him symptoms of cerebral involvement in malaria could mimic

those of cerebral tumour or very rarely disorders of extrapyramidal system; symmetrical haemorrhagic softening in the corpus callosum and internal capsule had been documented. He had also noted cranial nerves plasies especially occulomotor and facial besides, spinal cord disorders. Neurosthenic syndromes, paranoid, schizoid and manic depressive psychoses were also been described. Chadda et al (1979) have reported a case of smear positive malaria (P. falciparum) with high fever who presented with cerebellar signs.

Padmini et al (1980) have reported a case of peripheral neuropathy resembling Gullain Barre syndrome who on repeated smear examinations showed P. vivax infection and improved after antimalarial therapy.

Retinal haemorrhages occured in about 15% cases of cerebral malaria and exudates were rare (Kayembe et al, 1980).

Gopinathan et al (1982) have discussed 6 cases of cerebral malaria presenting with neuropsychiatric manifestations. Various degrees of impairment of orientation to time and place (rarely to person or self identity), memory impairment mostly for recent events, registration and recall and intellectual impairment causing clinical profile of confusion and consequent confabulation and abnormal behaviour was observed. In this series 5 cases were due to P. falciparum while one was caused by P.vivax.

In two cases onset was sudden, in one case it appeared during the therapy while in others these manifestations were observed five days after chloroquine therapy, suggesting resistance to therapy.

Warell et al (1982) and Davis et al (1982) have reported retinal haemorrhages in about 15% cases, whereas exudates were rare.

Gopinathan and Subramaniam (1982) studied 20 cases of cerebral malaria. Clinical features of these cases were classified as follows :

Pyramidal involvement	7 (35%)
Neck stiffness	7 (35%)
Urinary incontinence	5 (25%)
Apraxia	3 (15%)
Convulsions	3 (15%)
Hiccups	3 (15%)
Papilloedema	2 (10%)
Choreiform movements	1 (5%)
Cerebellar signs	1 (5%)
Facial paresis (unilateral)	1 (5%)
Associated renal involvement	3 (15%)

Sixteen of their cases were caused by P.falciparum, 2 by mixed infection and 2 by P. vivax. Prognosis was poor for patients with multisystem involvement and only 1 out of 3 cases survived. The patients having renal involvement showed heavy parasitemia and evidence of unconjugated hyperbilirubinemia.

Osuntokun (1983) was of the view that symptoms of central nervous dysfunction, especially cerebral are associated with a febrile illness; headache, muscle pains, vomiting, anorexia and diarrhoea. These are preceded by altered consciousness, convulsions, abnormal behaviour focal signs which are often transient, multiple and enavescent and sometimes signs of meningeal irritation are also present as the author has observed. He further asserts that in cerebral malaria, acute psychiatric disturbances including schizophrenic and manic like syndromes, depression of the exogenous orendogenous types, acute malignant anxiety, amok and confusional states, hallucination delirum, amnesia, twilight states could occur although an exact or casual relationship of these to malaria was at best tenuous.

In 1984, Chitkara et al reported cerebellar syndrome in children having malaria.

Loban and Polsok (1985) have summarized development of malarial coma. When malarial coma developed slowly three stages could be distinguished, namely, somnolence, precomatose state and true coma. Authors further observed that somnolence was related to apathy or excitement, negative attitude, disorientation, confused consciousness and drowsiness, sharp inhibition of all reactions to stimuli, including pain stimuli, intensification and then weakening of the tendon reflexes.

According to authors the precomatose stage was characterized by the following findings; pale face with greyish tinge and dry skin, the oral mucousa, sclera and conjunctiva were subicteric. There was tachycardia and the temperature reached 40-41°C. There was also hepatosplenomegaly, hypochromic anaemia, neutrophil leucocytosis with increased number of monocytes, eosinopenia, a high ESR proteinuria. Occassional ataxia, amnesia, convulsions, sometimes of epileptiform nature, progressive inhibition of deep sleep which could be interrupted for a short time only by strong tactile and sound stimuli. Occassionally there could be brief periods of semiconsciousness when the patients gave monosyllabic answers to questions and then rapidly reverted to stupor. The tendon reflexes were increased and pathological reflexes appeared. These authors stated that in true coma, both with slow and rapid development, the patient was unconscious, reacting to no external stimuli. He would be motionless, skin appearing pale or pale yellow sometimes with a greyish tinge. The eyes would be closed or half open and blank; there would be increased muscular tonus, trismus, rigidity of occiptial muscles, positive. Kernigs and Brudzinski signs; Babinski and Gordon signs could also be present. The tendon and abdominal reflexes could be absent and the vegetative functions severely disrupted. The pupils could be dilated, the pupillary reflex diminished and

disappearing altogether at the late stage of disease . According to authors following neurological manifestations could also occur with malarial coma : paseses and paralyses (monoplegia and hemiplegia), not infrequently convulsions (epileptiform), cerebral haemorrhages (seldom), dysarthria, aphasia and amnesia. Psychotic syndromes like delirium, manic states and hallucinations could also arise.

Ahmad et al (1986) in their study of 30 children with cerebral malaria found incidence of hemiparesis, monoparesis, ptosis and facial paresis (3.3% each) besides altered sensorium (100%) and convulsions (76%).

An expert committee of W.H.O. Malaria Action Programme (1986) has reviewed the clinical features of cerebral malaria. According to its report consciousness was impaired, which would be unarousable coma in strict terms. Neck rigidity and photophobia did not occur but mild neck stiffness was not uncommon. There ought to be no signs of raised intracranial pressure. Retinal haemorrhages and rarely exudates could also occur. The pupils would be normal. Disorders of conjugate gaze would be very common and the usual finding ought to be divergent eyes with normal oculocephalic and oculovestibular reflexes. Convergence spasm would be observed rarely. Consensual reflexes would be preserved unless patient was in grade IV coma. The jaw jerk would be brisk and pout reflex would be elicited. The gag reflex was usually preserved.

Muscle tone and tendon reflexes were often increased, but a general reduction in tone and reflexes would also be observed. Ankle and sometimes patellar colonus could be elicited and the plantar responses would usually be extensor. Abdominal reflexes were invariably absent. Decerebrate and decorticate postures could occur in severely ill patients. Extensor posturing could be associated with oculogyric crises and cyclical periods of stertorous breathing. Convulsions were common and were usually generalised without focal features. Agitation, confusion, transient paranoid psychosis or delirium could develop as sequalae. Other neurological sequalae included cranial nerve lesions, tremor and persisting coma but were unusual. Extraneural signs were common.

Above mentioned committee (1986) further observed that convulsions were common in children aged six months to five years who had high fever (more than 38.5°C) and it was difficult to differentiate clinically, convulsions caused by malaria from those caused by other febile illnesses. In one study in Thailand, convulsions associated with falciparum malaria occured in some 9.6% of children aged less than 5 years, but in only 1.5% of children with falciparum malaria aged 5 to 12 years (Changsuphajaisiddhi,T., personal communication to W.H.O. Malaria Action Programme, 1986). The neurological signs of cerebral malaria in infants and children were those of

symmetrical upper motor neuron and brain stem disturbances including dysconjugate gaze, decerebrate and decorticate postures. The committee has further stated that retinal haemorrhages and exudates could occur C.S.F. examination was usually normal but in some cases there was a slight increase in opening pressure and also increase in leucocyte count (mostly lymphocytes upto 50 cells/microlit.) and protein content (rately exceeding 150 mg/dl).

VIVAX MALARIA AS A CAUSE OF CEREBRAL MALARIA

In 1921, Rosale described lethal haemorrhage in the cerebellum of 21 year old soldier, during the course of vivax malaria.

Bystrone (1927), Tareev et al (1943), Nikolaev (1948) Osiovsky (1949) described cases of cerebral malaria due to P. vivax in Russian literature as quoted by Loban and Polozok.

Kitchen (1949) believed that serious complications in the course of vivax malaria could be due to an unvafourable premorbid background or intercurrent infection.

Mill et al (1963), while reporting case of vivax cerebral malaria, complicated by aphasia and hemipharesis, were of the view that P. falciparum parasitemia was probably missed in the presence of larger and more uniformaly distributed, P. vivax species.

Cerebral malaria caused by P. vivax multinucleatum had been described by Jiang et al (1965) in Yunan and Hunan

provinces of China.

Verma and Nagotra (1976) reported a few cases of cerebral vivax malaria in children residing in Jammu region.

In 1978, Bruce Chwatt expressed the view that in P. vivax infection cerebral malaria was rare.

Padmini and Maheshwari (1980) described a case of P. vivax malaria presenting as Gullain Barre syndrome.

Chabasse et al (1981) have opined on the rare occasions where cerebral malaria had been attributed to P. vivax; it was difficult to exclude inapparent mixed infection with P. falciparum.

Gopinathan et al (1982) reported a case of P. vivax malaria presenting with neuropsychiatric manifestations.

Gopinathan and Subramaniam (1982) in their series of 20 patients of cerebbal malaria found P. vivax infection in 2 cases and mixed infection (P. vivax with P. falciparum) in 2 other cases.

Ostuntokun (1983) kept only a rare possibility of cerebral malaria being caused by P. vivax.

Sachdeva et al (1985) have described 6 cases of vivax malaria out of which 4 died and one had transitory spastic hemiplegia.

Loban and Polzok (1985) maintained the view that earlier reported cases of cerebral malaria due to P. vivax in Russin literature were induced by mixed infection.

i.e. malaria and undiagnosed latent neuroviral infection (possibly hepetic), since the later stages of erythrocytic schizogony in case of P. vivax did not have any predilection for capillaries of internal organs and brain unlike that seen in case of P. falciparum.

Kidwai et al (1986) have reported 3 out of 11 cases and Ahmad et al (1986) have reported 4 out of 30 cases of cerebral malaria which were caused by P. vivax.

PATHOGENESES OF CEREBRAL MALARIA (A REVIEW OF VARIOUS THEORIES)

Marchiafava and Bignami (1984) observed at autopsy of fatal cases of cerebral malaria, brain capillaries filled with parasitised R.B.Cis even when parasitemia was low.

Gaskell and Miller (1920) noted presence of predominantly late trophozoites and schizonts of P. falciparum in brain capillaries and venuels - forms seldom seen in peripheral smears. Sticking together of these parasitised cells leading to impedence and stoppage of cerebral blood flow was suggested by the authors.

In 1921, Druck first described classical granuloma of cerebral malaria which presented as a proliferation of glial elements around ring sheped haemorrhages and perivascular necrosis.

Druck (1921) and Stern (1936) described malarial encephalitis, encephalomyelitis and even lymphocytic meningitis.

In 1941, Kinsley coined the term 'sludging' for the impedence of cerebral flow caused by sticking together of parasitized erythrocytes. Spitz (1946), Fischer and Reichenon (1952) noted occurence of pulmonary oedema and systemic circulatory failure in some cases of cerebral malaria.

Edington (1954) described characteristic ring haemorrhages and perivascular oedema and pericapillary infarctions in brain tissue.

Tella and Maegrith (1966) described release of vasoactive substances like bradykinin and bradykinogens from ruptured parasitised erythrocytes as an important pathogenic mechanism.

Edington (1967) made the observation that membranes of parasitized erythrocytes became changed and tended to agglutinate and stick together.

Dennis et al (1967) have established that a definite degree of clotting defect occured due to the insufficient utilisation of fibrinogen in falciparum malaria.

Borochevitz et al (1970) and Herzer and Greepel (1970), Reid and Nkrumah (1972) have noted disseminated intravascular coagulation (DIC) causing bleeding diatheses in cerebral malaria.

According to Schmid (1974) the basic and direct effects of malarial infections on the nervous system comprised of capillary blockage and damage by parasitized

cells which tended to form a peripheral layer closely adherent to the endothelium; micro infarctions with deposition of pigments in the tissues; pericapillary ring haemorrhages with or without evidence of narcotic arterioles.

Punyagupta (1974) and Srikaichul (1975) reported (DIC) in severe falciparum malaria and reemphasized its role in the pathogenesis of cerebral malaria.

Reid (1975) denied any importance of fibrin in the pathophysiology of malaria and warned against the wide use of heparin like anticoagulants. Maegraith (1976) also did not consider DIC as a significant pathogenetic mechanism in cerebral malaria.

Vietze (1978) found polypeptides in the blood of patients suffering from malaria which are known to inhibit oxidative phophorylation in mitochondria. This led the authors to consider the possibility of anoxic oedema in the pathogenesis of cerebral malaria.

Balonieil et al (1980) considered hypoxemia caused by pulmonary oedema as a contributory factor in the pathogenesis of certain cases of cerebral malaria.

Ogtuntokun (1983) has reviewed the pathogenesis of cerebral malaria in detail. He rules out the possibility of true encephalitis in malaria. He considers that systemic circulatory failure and pulmonary oedema could occur in severe malaria infections. [illegible] could be an additional

mechanism in cerebral malaria which lowered cerebral perfusion as well as caused cerebral hypoxia.

Chanthavanich et al (1983) did not find cerebral oedema as a consistant feature in patients of cerebral malaria.

Usawattanakul (1985) detected endotoxin in patients with cerebral malaria and found that it was not related to clinical syndrome.

Seidel (1985) expressed the view that pathogenesis of cerebral malaria was result of cytotoxic anoxia caused by parasitized erythrocytes in microcirculation.

According to Macpherson et al (1985) the essential pathological feature of severe falciparum malaria was the sequestration of erythrocytes containing mature forms of parasite in deep vascular bed. Sequestration was greatest in the brain.

THE PERMEABILITY THEORY

Rigdon (1942) working on Macaca rhesus monkeys infected with P. Knowlesi found increased permeability of blood brain barrier.

Tella and Maegraith (1966) reported release of certain vasoactive substances like bradykinin and kinogen from ruptured parasitized R.B.C's which could increase the capillary permeability.

Migasena and Maegraith (1967) observed that increased permeability was reversed rapidly by hydrocortisone, mepacrine and chloroquine.

Angus (1971) noted release of free fatty acids which could enhance capillary permeability.

Maegraith and Fletcher (1972) described the vasoactive substances released from parasitized erythrocytes including kininis, kallikrein, kinenogenases, histamine and adenosine peptides. According to his theory the primary pathophysiological abnormality in cerebral malaria was an increase in cerebral capillary permeability with outward leakage of plasma. This was considered to result in cerebral oedema and because of the extravasation of plasma into the cerebral interstitium, local haemoconcentration and reduced micro circulatory blood flow occured.

A committee of W.H.O. Malaria Action Programme (1986) did not find this theory based on animal studies consistent with observations made in human cerebral malaria. The committee favoured outright rejection of this theory.

MECHANICAL THEORY

This theory simply states that the pathophysiology of severe falciparum malaria should be explained by microcirculatory obstruction with consequent local hypoxia and substrate depletion i.e. ischaemia (W.H.O. Malaria Action Programme, 1986).

Miller et al (1972) showed that cells containing P. Knowelesi did not pass through micropore filters as easily as unparasitized cells.

Kilejian et al (1977) noted the formation of knob like protrusions from the parasitized erythrocytes which by antigen affinity could attach themselves to the capillary endothelium and phagocytes resulting into formation of lumps and aggregates.

According to Udainya et al (1981) infected erythrocytes developed knob like protrusions by which they attached to endothelium through specific recepter ligand interaction.

Jeandel et al (1982) commenting on the knob like protrusions from parasitized RBCs and their adherence to capillary endothelium, hypothesized a local vasculopathy as the main factor in the pathogenesis of cerebral malaria.

Leech et al (1984) pointed out these knob like protrusions from the erythrocyte surface overly accretions of parasite derived antigen.

Cranston et al (1984) demonstrated that P. falciparum infected erythrocytes showed reduced deformability and that this was directly proportional to the maturity of the intracellular parasite.

Macpherson et al (1985) observed that parasitized RBC's adhered to endothelium via surface knobs.

An expert committee of WHO Malaria Action Programme (1986) did not consider reduced deformability of parasitized RBCs as an important pathogenic mechanism for microcirculatory obstruction. However, the committee hypothesized selective adhesion of parasitized erythrocytes to endoth-

elium via knob like protrusions to specific receptors presumably more abundant in brain vessels.

IMMUNOLOGICAL THEORY

This theory holds some form of immunologic mechanism responsible for the pathogenesis of cerebral malaria (reviewed later).

COMPLEMENT

The name complement stands for a highly complex multimolecular self assembling biologic system that constitutes one of the major humoral mediators of inflammation and participates in host defence (Nusinow et al, 1985).

HISTORY

The discovery of complement system dates back to 1894 when Pfeiffer demonstrated that the immune system of guinea pigs acquired the capacity to dissolve cholera bacilli (Pfeiffer's phenomenon).

Bordet (1896) identified a heat labile factor in both immune as well as non immune serum besides a heat stable factor present only in immune serum.

Buchner named this heat labile protective activity of blood as 'Alexin'.

In 1920's there were 4, in 1960's 9 components were known (one of which had 3 subcomponents) C_3 fraction was first isolated by Muller Eberhard in 1960.

At present the complement system is recognised to consist of atleast 20 separate proteins (14 complement proteins and 6 regulation proteins) that circulate in blood as inactive precursor molecules (Muller Eberhard et al 1976-77).

General properties and nomenclature (Nusinow, 1985) :

The human complement system consists of more than 20 plasma proteins that are chemically, functionally and immunologically distinct (Muller Eberhard H.J., 1975). The proteins are labelled as components (c) and designated by consecutive numbers in the order of their discovery (C1, C2, C_3 etc.). Some of them are labelled by the name Factor which is suffixed by a letter viz. Factor B, Factor D, Factor I (C_3 b inactivator), Factor H (B1H globulin). Some of the names of regulator proteins are often related to the function of the protein as in the case of C1 inhibitor, C3b inactivator or C4 binding protein. The presence of a bar over a component indicates an active enzyme, as in the case of $\bar{C}1$ or $\bar{D}$. The presence of a small letter after the number of letters of a complement protein indicates a fragment derived from the cleavage of the parent complement component. For example, the activation of C3 produces two fragments (1) the C3a anaphylatoxin and (2) C_3b, the fragment associated with opsonisation.

Table (1) below shows the properties of various complement components (Turner, 1983).

Table - I

After Turner, 1983 and Musinow, 1985)

Component	Serum Conc. Microgm/ml.	Mol. Wt.	Substrate cleaved
Classical pathway			
Early components			
C 1 q	150	410000	
C 1 r	50	160000	C 1s
C 1 s	50	83000	C4, C2
C 4	400	206000	
C 2	15	110000	C3, C5
C 3	1200	180000	
Alternative pathway			
Early components			
D	5	24000	B
B	2000	93000	C3, C5
C 3	1200	180000	
P	25	204000	
Terminal components			
C 5	80	200000	
C 6	70	120000	
C 7	65	120000	
C 8	80	154000	
C 9	200	79000	
Central proteins			
C 1 inhibitor	200	110000	
1 (e3b inactivator)	20	1000000	C3b, C4b, C5b
H (B_1H globulin)	650	150000	
Serum carboxy	35	-	
Peptidase			
Properdin	25	-	
C4 binding protein	-	-	
S. protein	-	73000	

Sequence of Activation :

According to Yachin et al (1966) complement system is an important mediator of immunological and inflamatory reactions and can be activated by immune complexes or by non immunologic mechanism such as proteolytic enzyme of the coagulation system. This view was further strengthened by Osler et al (1973) and Arroyave et al (1977).

Activation of complement system is generally achieved as a result of proteolytic cleavage by the preceding component and usually reveals an enzymatically active site which will in turn act on a latter component (hence cascade). Activated component usually has very short biological halflife and will decay to an inactive form if subtrate molecules are not encountered.

In addition, there are several regulator proteins which play a critical role in protecting host tissue against the potentially damaging effects of uncontrolled complement activation. These inherent mechanisms in the system permit both rapid activation and rapid shut. Two pathways of complement activation are recognized, the so called classical and alternative pathways (Nusinow et al, 1985).

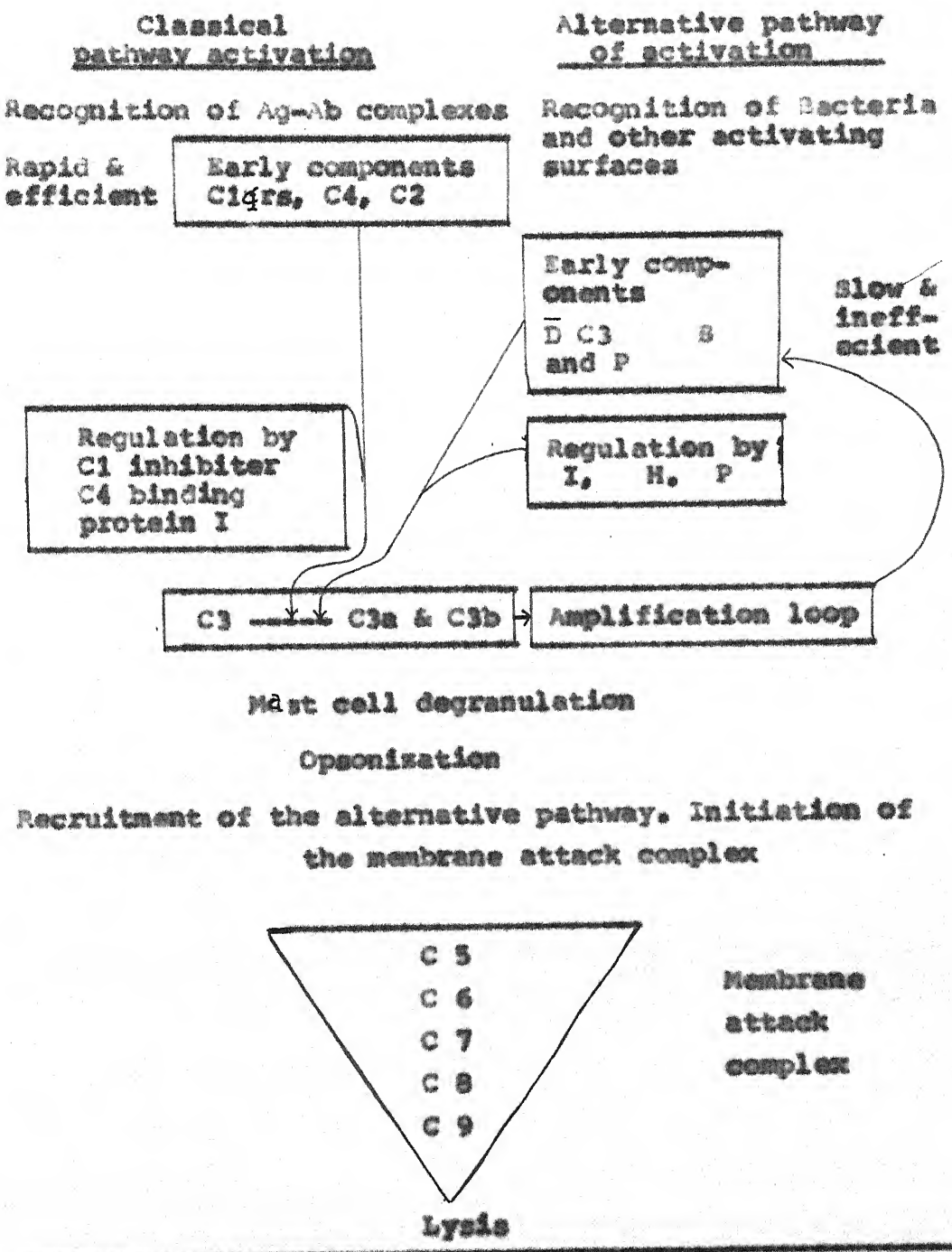


Diagram : Components of the complement system (after W E Paul (1984) and S.R. Husinow (1985)

The salient features of complement activation are the classical and alternative pathways which interact with each other and yield enzymes (convertases), able to clear C 3 and C 5.

In the classical pathway complement activation occurs in the order, Antigen-Antibody-C1, 4,2,3,5,6,7,8 and 9. (Asten et al 1968). In the alternative pathway it occurs in the order, Activator-propardin system (B,D,P,C3) C3, 5,6,7,8,9 (Getzeet al, 1971, Gewurzs et al, 1972).

Activators of the classical pathway include aggregates of Ig G and Ig M or immune complexes, C-reactive protein certain lipopolysaccharides (endotoxins), and some viruses (Nusinow et al, 1985). On the other hand, activators of alternative pathway are rabit erythrocytes, gram negative bacteria, agregates of IgA and certain B lymphocytes (Nusinow et al, 1985).

The regulatory proteins of the classical pathway include C1 inhibitor, C4 binding protein, C3b inactivator (I) and S-protein. The blood also contains an inactivator of the anaphylatoxins C3a, C4a, and C5a named serum carboxypetidase B or N. The regulator proteins of alternative pathway are : factor I and H (B-IH globulin) as well as properdin (Nusinow et al, 1985).

Common final pathway of the complement system forms the membrane attack mechanism and leads to lysis of offending organism (Haynes and Paci, 1987).

Quantitation of complement system :

Immunodiffusion is an important precipitin test used for the quantitative assessment of complements in the serum.

Oudin (1946) described a single dimension precipitin test. In this technique antigen is allowed to diffuse through gel containing antibody placed in a convenient sized tube. A band of precipitate forms at the zone of equivalent concentration.

Elek and Ouchterlony (1948) published their double dimension technique. The authors described that when antigen and antibody were placed in separate wells cut in the gel at a suitable distance from each other and were allowed to diffuse, various types of precipitin lines were formed at the zone of equivalence.

Oakley and Fulthrope (1953) described a double diffusion single dimension system. In this method, a zone of neutral agar was placed between antigen and antiserum in a tube.

Feinberg (1959) first developed single diffusion double dimension technique of radial immunodiffusion which was later modified by Mancini (1963). Authors observed that antigen diffused radially from the point of application into a gel containing antibody and a circular precipitate was formed at the zone of equivalence. The diameter of precipitin ring was proportional to the concentrations, provided that gel thickness remained constant. Authors

allowed antigen to diffuse until the precipitate ring stopped enlarging.

Fahey and Mackelvey (1965) further modified radial immunodiffusion technique. They measured the diameter of the precipitan ring at the fixed time between 18 and 20 hours.

Biological importance of complement :

The biological activities of complement components in immunological and inflammatory response are given in the following table (Turner 1983 and Ruddy 1985).

Complement	Activity
C 1	Stabilization of Ag-Ab complexes.
C4b	1. Neutralization of virus infectivity.
	2. Immune adherance to lymphocytes & phagocytes.
C2b derived fragment.	Kinin activity - increases vascular permeability.
C3a	Anaphylatoxin; evokes histamine release from basophils; potent chemoattractant for monocytes and neutrophils.
C3b	1. Major opsonin. Binds to specific receptor on neutrophils, eosinophils and macrophages.
	2. Binds to B lymphocytes. Modulates immune response.
	3. With Bb forms alternative pathway C3 convertase and amplifies alternative pathway.
	4. Promotes solubilization of immune complexes.
C3d	Mediates immune adherence through binding to a specific receptor on macrophages.
C5a	1. Anaphylatoxin. 2. Chemotactic factor.
C5b67	Chemotactic factor
C8	Low grade membrane damage.
C9	Rapid membrane damage.

Role of complement in elimination of immune complexes (IC's):

There are different complement mediated processes which cooperate in immune complex (IC) elimination.

According to Gigli et al (1968) and Ruddy et al (1972), the ICs bearing C3b on surface, were capable of binding to the C3b receptors of polymorphonuclear leukocytes and also to the cells of mononuclear phagocyte system. Thus IC's were finally phagocytosed. Miller et al (1975) reported that C3b in the immune complexes could change the conformation of the complex itself. As a result large complexes were split into smaller ones. These smaller complexes were unable to deposit in the tissues and got ultimately detoxified.

According to Haynes and Fauci (1987) the immunoglobulin isotype composition is a critical factor in determining complement activation and therefore in determining effeciency of clearance of IC's by Fc receptor bearing cells within the immune system. Immune complexes of IgM/IgG activate rapid and efficient classical pathway while IC's of IgA activate slow and inefficient alternative pathway.

IMMUNE COMPLEXES

An immune complex is the one produced by interaction of antigen and antibody which may or may not be complement fixing (W.H.O., 1977).

Immune complexes - a brief history :

For long, role of immune complexes in host defence was known. It was only in the early part of this country (1911) when Von Pirquet hypothesized their role in certain human diseases. Fifty years later (1951) he and his associates presented an experimental model of serum sickness i.e. an immune complex disease. His observations regarding role of circulating immune complexes (CIC) were later confirmed by Germuth (1953) and Dixon (1958). Since then a number of diseases have been attributed to immune complexes.

Circulating immune complexes (CIC) in health :

Isenberg et al (1981) and Jans et al (1982) have observed circulating immune complexes in normal healthy individuals. According to Endo et al (1985) C.I.C. are not necessarily pathogenic. They represent a physiologic mechanism forthe removal of exogenous and endogenous antigens and are usually eliminated without resulting in tissue injury. They further described their detection in normal subjects, a circadian and seasonal variation and variation with food ingestion and exercise.

According to Lawley and Frank (1987) larger complex are rapidly removed from the circulation in the liver. Complement activation seems to be an important mechanism which cleaves larger complexes into smaller ones which are inturn detoxified by liver reticuloendothelial system. They have also highlighted role of erythrocytes which acting via

complement receptors attack immune complexes and during their sequestration are stripped off these complexes in the hepatic sinusodies.

Role of immune complexes in diseases :

Formation of immune complexes can occur under following circumstances (W.H.O., 1977) :

1. Antibody reacting with antigen present as a part of a membrane either integral or passively attached. This is the type II allergic mechanism of tissue damage. Antibody that has Bound to cell membrane components can be shed secondarily as a complex, from the membrane into fluid phase.

2. Antibody reacting with soluble non cell bound antigen. This is the type III allergic mechanism of tissue damage. The consequences of this type of reaction vary with the location of complex formation.

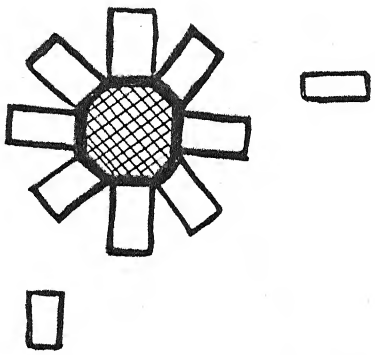
W.H.O. Scientific Group (1977) further notes that complexes can be formed :

(a) When both antigen and antibody are blood borne and secondarily localise in blood vessels and perivascular tissues (e.g. in serum sickness).

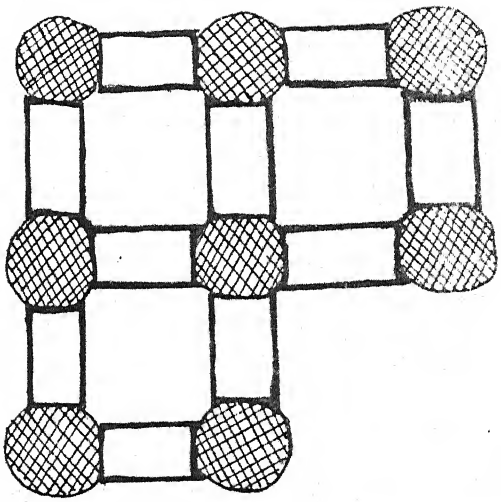
(b) When antigen locally released in the tissues reacts with blood borne antibody (e.g. in onchocerciasis) and

(c) When both antigen and antibody are formed locally (e.g. late granulomas around schistosome eggs).

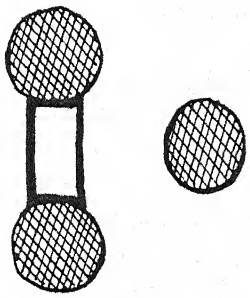
LATTICE THEORY OF MARRACK DEPICTING ANTIGEN ANTIBODY COMPLEXES



ANTIBODY EXCESS



EQUIVALENCE



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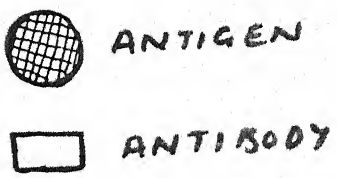

(AFTER. J.A. LAKIN - 1980)

FIG-3

Size of immune complexes (Fig. 3) :

According to Lawley and Frank (1987), the size of the circulating immune complexes is an important parameter of toxicity. In general larger (> 19S) complexes cause more tissue damage, than do small complexes. The authors further state that the size is related to the concentration and molar ratio of antigen and antibody, as well as to the avidity of the antibody for the antigen.

In antibody excess, antigen valences are saturated and in general the complexes are small. Under conditions of antigen excess, antibody combining sites are saturated, chances for lattice formation are limited and again the complexes are small. At equivalence or mild antigen excess, lattice formation, is facilitated and large complexes can form. Immune complexes formed at moderate antigen excess are thought to be most pathogenic, perhaps because they are most efficient at activating the various mediator systems like complement cascade.

Deposition of circulating immune complexes :

According to WHO Scientific Group (1977) most of the CIC's are cleared by mononuclear phagocyte system, particularly Kupffer cells. This applies especially to large complexes and those that are complement fixing. Complexes that are smaller, or non complement fixing are cleared to some extent by spleen, or they may become fixed to the renal glomeruli, blood vessels walls or choroid plexus.

Endo et al (1985) consider vascular bed as an important variable when considering possibility of immune complex injury. Immune complexes tend to be deposited in organs with specialized vasculature, like the kidneys, skin, joints, choroid plexus and arterial walls. They further opined that once immune complexes are deposited in tissues, injury may ensue only if the permeability of local vessels is increased by the local release of vasoactive amines. Complement activation by immune complexes may promote the release of vasoactive amines.

Mechanisms of tissue injury by immune complexes :

Endo et al (1985) have summarized various mechanisms of tissue injury as follows :

1. Activation of complement resulting in :
   - Immune adherence
   - Chemotaxis
   - Cytoxicity
   - Immune complex solubilization
   - Release of leukocytes from bone marrow
2. Platelet aggregation and release of their vasoactive amines.
3. Neutrophil phagocytosis and degranulation.
4. Macrophage phagocytosis
5. Basophil and mast cell degranulation
6. Suppression of B cell by Ig G immune complexes
7. Enhanced humoral response.
8. Activation of T suppressor cells by IgG immune complexes.
9. Stimulation or inhibition of T helper cells
10. Enhanced or depressed killer cell activity

STUDY OF COMPLEMENT AND IMMUNE COMPLEXES IN MALARIA AND CEREBRAL MALARIA

Cathoire (1910) and Vincent (1910) first noted the depression of serum complement in human malaria.

In 1939, Eaton found a soluble antigen in the serum of monkeys heavily parasitized with P. knowlesi. Injected into normal monkeys it gave rise to complement fixing antibodies, but they conferred no protection against plasmodial challenge.

Again in 1948, Dulaney et al found that the serum of most patients with induced malaria had diminished complement activity.

Fogel et al (1966) and Cooper and Fogel (1966) made a detailed study of the effects of plasmodium knowlesi infection on complement activity in Rhesus monkeys (Macaca mulatta). Nearly all monkeys with heavy parasitemia had depressed total haemolytic complement levels. Initially, a cyclical variation in complement activity was observed, complement levels falling in association with merozoite release. In the terminal stage of the infection a marked fall in serum complement occurred. Complement components C 1, C 2 and C 3 were all depressed.

Cox (1966) detected in the serum of monkeys actualy infected with P. knowlesi, an antigen which reacted in gel precipitation tests with sera from animals convalescent from malarial infection.

Berger (1967) reported nephrotic syndrome secondary to, falciparum glomerulonephritis and thought of possible immunological basis.

Wright (1968) noted that experimental neonatal thymectomy in golden hamsters infected with Pl. berghei almost suppressed the development of acute haemorrhages of the brain due to an intravascular antigen-antibody reaction. He also observed low levels of complement in hamsters with cerebral malaria.

Mc Gregor et al (1968) conducted their experiments over sera and plasma of Gambian children. They found soluble malarial antigen in patients either suffering from P. falciparum malaria or recovering from infection. Antibodies were detected in adults, but were rare in children under 6 years of age. They also noted that the antibodies were not detectable once circulating soluble malarial antigens were eliminated. Basing their views on these observations authors opined that soluble antigens were weakly immunogenic. Authors were unable to find any evidence of harmful effects of these antigens, contrary to suspicions of Dixon (1966) that immunopathological sequences may sometimes follow the deposition inh host tissue of malarial antigens or Ag/Ab complexes.

Allison et al (1969) found evidence of soluble complexes which got deposited in kidneys along the basement membrane on the epithelial side of Bowman's capsule in

P. malariae infection. But they did not get any evidence of P. falciparum infection in such cases.

Ward et al (1969) studied the nephrotic syndrome due to P. malariae infection in east African children. They found evidence of glomerular deposits of Ig M, IgG, Ig A, complement and fibrin, sometimes with malarial antigen. They were of the view that this deposition was secondary to soluble immune complexes.

Wilson et al (1969) while studying malarial antigens and respective antibodies in Gambian population constantly exposed to P. falciparum infection found four varietes of soluble antigens viz. S (stable to 100°C), R (resistant to 56°C) and La and Lb (labile to 56°C). They detected only transient antibody response to antigens in infected children. La antigens produced antibody response in early childhood and could be detected in virtually all individuals above 6 years of age. Lb antigens stimulated antibody production in a small percentage of adults and adolescents. Authors noted that with heavy parasitemia S-antigen was found in increased concentrations while La antigen was barely detectable. They suggested two explanations for this discrepancy. First, the antigen (La) might be relatively insoluble, hence not circulating in body fluids. Second, the antigen might be rapidly complexed in-vivo by the specific antibodies which were demonstrable in most individuals.

Wright et al (1971) showed that injection of antithymocyte serum to P. berghei infected golden hamsters and rats suppressed the intravascular antigen-antibody reaction and thus the development of acute haemorrhages in brain. He also noted hypocomplementemia in experimental cerebral malaria.

Houba and Williams (1972) found evidence of circulating immune complexes while conducting their study on soluble malarial antigens of P. falciparum in Nigerian population.

Bhamarapravati et al (1973) studied ten cases of P. falciparum infection which showed urinary abnormalities. Kidney tissue showed deposition of immunoglobulins (Ig G, Ig m and Ig A) and complement in glomerular basement membrane and mesangial areas in all but one case. Malarial antigen was detected in two cases. Sera of two patients who had evidence of circulating immune complexes showed depressed levels of C3 and C4 suggestive of activation of classical complement pathway. The authors concluded that immune complex nephritis could occur in P. falciparum malaria.

Rosenburg et al (1973) demonstrated in patients with P. falciparum malaria infection that the degree of anaemia correlated well with rising titres of Ig M antierythrocyte antibodies and decreased levels of C3. They proposed an autoimmune mechanisms for anaemia in which complement activation played a role, hence the decreased levels of complement.

Greenwood and Brueton (1974) reported low C3 levels, very low C4 and C1q levels and relatively normal glycine rich B glycoprotein (GBG) in sera of most children with acute falciparum malaria. Their study suggested activation of complement by classical pathway. They further opined that lowering of C3 level was probably due to formation of antigen-antibody complexes with soluble malarial antigen leading to activation of classical pathway. They did not find any correlation between the initial C3 level of children with cerebral malaria and the duration of their impaired consciousness. But they did find a correlation between detection of soluble malarial antigen and significantly lower C3 levels. These authors found a positive correlation between raised fibren degradation products (FDP) levels and low serum C3 levels in children who had neurological signs. According to authors this finding further suggested complement activation by Ag-Ab immune complexes leading to vascular damage in severe falciparum malaria.

In humans with plasmodium vivax infection low levels of serum complement were demonstrated by Neva et al in 1974. They linked complement depletion to schizont rupture and found a direct correlation between low levels of CH_{50} and C4 with the degree of parasitemia and also to the presence of complement fixing antibody. Also they suggested activation of complement system by malarial antigens leading to depletion of complement components.

Srikaichul et al (1975) found a positive correlation between the reduction of C3 on one hand and clinical complications as well anaemia and thrombocytopenia on the other hand. The patients with falciparum malaria. They further observed more severe reduction of C3 levels in most severe cases of thrombocytopenia in the patients associated with disseminated intravascular coagulation (DIC). The more rapid loss of radiolabelled C1q in parasitemic patients was interpreted as a reflection of C1q binding to immune complexes formed in malaria. The authors opined that complement activation leading to disseminated intravascular coagulation and promotion of release of vascular permeability factor could be considered as an important pathogenic mechanism in complicated falciparum malaria.

According to a scientific group of WHO (1975) (quoting Lambert and Mouba, 1974), in owl monkeys infected with P. falciparum or with P. brasilianum, an increase of C4, C3 and properdin factor B or (C3PA) was observed during the peak parasitemia. It was followed by a decrease of these three components far below their normal range.

This group also quoted unpublished data of Krettli et al who, in mice infected with P. berghei, found an increase in the level of C3 during the first three days of infection which progressively decreased after fourth day of infection, becoming undetectable in second week of

infection. Since the death from cerebral malaria was most frequent in the preschool children this scientific group suggested an allergic response to malaria in such patients as patients of more than one year of age had appreciable level of malarial antibody.

Houba et al (1976) experimentally demonstrated circulating immune complexes in one monkey infected with P. brasilianum which could be the result of interaction of circulating malarial antigens with antibodies. They noted that circulating immune complexes were deposited in various vascular territories. They showed presence of specific malarial antigen (P. falciparum) in deposited immune complexes in infected owl monkeys.

Petchelai et al (1977) studied complement changes in 31 cases of acute falciparum malaria. They noticed a considerable reduction in C3, C4 and C6 levels in complicated group and a similar but lesser reduction was found in the non complicated group while raised levels of C1q, C3BA, C8, Ca were found in both groups. Increased levels of C1q, C3PA C8 and C9 were explained on the basis of acute phase response which could mask utilization. Activation of classical complement pathway alone was found in seven cases. In another 2 cases activation of both classical and alternative pathways were found.

Toro and Roman (1978) interpreted neuropathological findings in cerebral malaria as resulting from a hyperegic

reaction of the CNS to the antigenic challenge of P.falciparum infection. They proposed an immune complex vasculitis as the pathogenic mechanism.

Greenwood et al (1978) studied the role of immunological factors in the pathogenesis of anaemia of acute falciparum malaria in children. Serum levels of immune complexes were normal at the time of presentation and increased only one month later. Low levels of C3 and C4 were detected but only in nonanaemic patients. These workers ruled out the immunological mechanism for anaemia in such cases.

Weis (1978) detected deposits of immune complexes in the lungs of mice infected with P. berghei.

Williamson (1978) reported hypocomplementemia in children with P. falciparum malaria.

Woodruff et al (1979) showed association of complement containing immune complexes on the rd cells surface with their decreased life even after complete eradication of malarial parasites.

Facer et al (1979) suggested a type III immune complex mediated hypersensitivity, involving parasite-antigen-antibody complexes, to explain coombs positivity and sensitization of erythrocytes with complement (C3d and C4b) and immunoglobulin G, leading to anaemia in children suffering from falciparum infection.

Perrin et al (1979) observed malarial antigens in serum of patients with acute falciparum malaria which showed a peak before therapy started and rapid decrease after therapy. Specific malarial antibodies became detectable 5-7 days after starting treatment in patients with first infection. Immune complexes were detected in sera of 21 out of 23 patients with peak levels between days 5 and 9. A marked decrease of C3 and C4 was also observed by the authors with normal levels of factor B.

According Houba et al (1979) in acute falciparum malaria circulating immune complexes could localize in glomeruli and initiate kidney lesion which was reversible and responded to therapy.

Boonpucknaving et al (1979) demonstrated granular deposits of immune complexes in all glomeruli of mice infected with P. berghei and they developed diffuse proliferative glomerulonephritis.

June et al (1979) studied circulating and tissue bound immune complexes in mice infected with P. berghei. The most significant finding was the presence of tissue bound immunoglobulins in the chroid plexus besides glomerular deposits. The appearance of antimalarial antibodies and malarial antigens in the serum was closely associated with a depression of C3 levels and presence of circulating immune complexes (CIC). High levels of CIC were found in primary infection even at relatively low parasite levels. The authors suggested a relevance of tissue bound immunolobulin in choroid plexus to pathogenesis of cerebral malaria.

In 1980 Contreras et al described development of circulating immune complexes in association with marked depression of C3 levels in various strains of mice infected with P. berghei.

Ehrich et al (1981) while experimentally producing transient glomerular injury resembling human P. falciparum infection in rats infected with P. berghei noticed transient elevation of circulating immune complexes and persistent antiplasmodial antibody in serum. They also showed tissue bound immune complexes in glomerulei on later examinations.

Ade-Serrano et al (1981) showed hypocomplementemia (C3 and C4) in Nigerian children suffering from P. falciparum parasitemia. Hypocomplementemia was greater when forms other than gametocytes were present.

Adams et al (1981) detected circulating immune complexes (CIC) and marked hypocomplementemia in patients with cerebral malaria (CM). CIC were rare and hypocomplementemia was not marked in patients with uncomplicated falciparum malaria. In 7 of the 9 patients of CM shortly after quinine therapy was initiated, there was a marked increase in cryoglobulin and CIC levels, associated in four instances with increase in the severity of the coma. Deepening of coma was explained on the basis of deposition of immune complexes in choroid plexus. Authors further put the possibility of CIC causing pathological

manifestations without being deposited in tissues, i.e. complement activation involving (i) liberation of vassoactive peptides with potential shock inducing activities and (ii) platelet activation and initiation of blood coagulation.

According to Idris Moh. (1982) some of the acute manifestations of cerebral malaria were remainscent of immune complex mediated disease. He postulated that during acute P. falciparum infection, the non immune patient was unduly suscepitible to rapid formation of antigen antibody complexes, which being complement fixing tended to deposit in the brain, as well as in other tissues.

Gupte et al (1982) reported low levels of CH_{50} and C3 in serum of patients with malaria which did not bear any correlation with parasite index.

Macrophage receptors for opsonized plasmodia were blocked by immune complexes in vitro (Brown and Krier, 1982). The authors concluded that immune complexes in the serum of actualy infected mice could protect the plasmodia from the activities of macrophages.

Finley et al (1982) noted presence of higher levels of CIC and lower levels of serum C3 in mice infected with P. berghei when its immune mechanism was intact. Cerebral malaria which developed in such mice was more severe as compared to those where immune mechanism was not intact (T. cell dependent mice).

Drawing conclusion from his experimental study, on P. berghei infected mice, Shear (1984) suggested that immune complexes modulate the immune response to malaria by inhibiting immune phagocytosis and perhaps by interfering with other effector mechanisms.

Macpherson et al (1985) did not find any evidence of immune complexes in cerebral vessels of patients with cerebral malaria at autopsy.

Phamuphik et al (1985) noted decreased levels of serum complement components in patients with P. falciparum malaria; C1q, C3 and C4 were most significantly reduced. Decrease was more profound in complicated cases with cerebral, renal and hepatic involvement. But they did not find any correlation between hypocomplementemia and the degree of parasitemia or the level of circulating immune complexes.

Kidwai et al (1985) observed hypocomplementemia in patients with malaria. C3 was lesser in falciparum than in vivax malaria but C4 levels were independent of species.

Sachdeva and Man Mohan (1985) found evidence of circulating immune complexes in two children, with vivax malaria who also showed profound decrease of C3 levels. These two children died despite treatment.

Kidwai et al (1986) and Ahmad et al (1986) noted an inverse correlation between pretreatment serum C3 and C4 concentrations and the neurological involvement. They further noted that very low C3 and C4 levels were bad prognostic indicators in cerebral malaria in children.

MATERIAL AND METHODS

MATERIAL AND METHODS

The present study was conducted in the department of Paediatrics, M.L.B. Medical College, Jhansi over a period of one year. The study included twelve patients of cerebral malaria and nine healthy controls. The cases were selected as follows :

A. Healthy controls :

Children belonging to the hospital staff, sibs of patients attending Paediatric out patient department or admitted in the ward were picked up as healthy controls. These cases were examined especially to exclude clinical infections and allergic disease. The age groups of selected cases ranged from 2 years to 8 years, corresponding to those of patients.

B. Children with cerebral malaria :

All the patients admitted in the Paediatric department with suspected cerebral malaria were subjected to peripheral smear examination by using Giemsa stain. Twelve patients in the age groups of 1½ to 8 years with asexual forms of malarial parasites in their smears and those in an unarousable coma were selected for the study. To exclude post ictal coma patients having a deep coma of more than 6 hours duration following convulsions as described by Warrell et al (1982) were included in this study. To exclude other causes of coma/encephalopathies following points were specifically noted in the history and clinical examination :

1. History :

History of fever and its details viz duration and relationship to unconsciousness were noted. History of convulsion, its nature and relation to height of fever and any past history of convulsion with or without fever was especially recorded to exclude the possibility of febrile seizures. History of vomiting, loose motion, headache and/or haemoglobinuria was noted. Any past history of malaria was recorded. Perinatal and developmental history was also noted.

2. Physical examination :

Patients were examined to determine the grade of unconsciousness, focal neurological deficit, superficial and deep jerks, abnormal movements including convulsions and signs of meningeal irritation. Presence of pallor, jaundice, hypotension, pupillary size and reaction were also recorded. Besides, examination of other systems was carried out. Size of the liver and spleen was specifically noted. Any evidence of bleeding episode was thoroughly searched.

A complete record of treatment given to the patient and progress, following treatment was kept.

Collection and storage of blood samples :

About 5 ml of blood was collected by venipuncture from every patient after correction of any dehydration/ hypovolemia. One ml of blood was kept aside for total

and differential leukocyte counts, Hb, E.S.R. and P.C.V. measurements. Four ml blood was used for the separation of serum which was preserved in a sterile vial at -20°C within 30 mts. to 1 hour of bleeding. Stored sera was thawed only once i.e. at the time of complement study.

Determination of C3 and C4 :

Complement components C3 and C4 were quantitated in patients sera and circulating immune complexes (polythylene glycol precipitates). Circulating immune complexes were separated as described later.

C3 and C4 were measured by single radial immunodiffusion using commercially available immunodiffusion plates. Reference serum provided by the manufacturer, having known quantities of C3 and C4 was filled in four wells in the concentrations of 100%, 75%, 50% and 25%. Reference serum was diluted by using autopipettes. Other wells were filled with patients sera, controls sera, prepared solution containing circulating immune complexes (PEG precipitates) of patients and controls. Each well was filled with 5 microlit of the test material. After filling the wells, plate was kept aside for 10 minutes to dry and then incubated at room temperature in humid atmosphere in an inverted position for 72 hours. Diameter of each precepitin ring was measured soon after 72 hours incubation (Mancini's method) were complete, to the nearest 0.1 mm. Diameter square of the reference sera was used to plot

standard graph. Against this standard graph values of unknown samples, corresponding to their diameter squares, were read. These readings were taken as levels of C3/C4 in the sample after correction of dilution factor which was used only in cases of controls sera (1:1).

Separation of circulating immune complexes :

Circulating immune complexes were precipitated from sera of patients and controls by polyethylene glycol (M.W.6000) precipitation method as described by Chia et al (1977). 1 ml serum of each case was taken in a tube and 1 ml of 8% polyethylene glycol in phosphate buffer saline (PBS) (pH 7.4, 0.01 M) was added to it. Contents of the tube were mixed by lateral shaking. The test tubes were incubated at 4°C for 18 hours and then centrifuged at 4°C over 1 hour at 1000 G using a refrigerated centrifuge. The precipitate so obtained was in the form of a pellet which was thoroughly washed with cold 4% polyethylene glycol in phosphate buffer saline (pH 7.4, 0.01 M). The pellet were then dissolved by shaking in 1 ml phosphate buffer saline (pH 7.4, 0.01 M) and solution was kept for estimation of C3 and C4.

OBSERVATIONS

OBSERVATIONS

The present study was conducted in the department of Paediatrics, M.L.B. Medical College, Jhansi over the period of one year from Aug. 1987 to Aug. 1988. The study group comprised of 12 children with proved cerebral malaria (Warrell et al, 1982). Age of children in the study group ranged from 1½ years to 8 years. The control group consisted 9 healthy children free from infection, allergic disorder or any other disease that could have led to alteration in the immune status of the child. Ages of controls ranged from 2 years to 8 years.

1. Age distribution

Age distribution of patients and control cases is shown below in table - I.

Table - I

Clinical group	Age in years			Total
	1 - 4	4 - 7	8	
Cerebral Malaria	1	9	2	12
Normal controls	2	5	2	9
Total	3	14	4	21

Nine cases of cerebral malaria fell in the age group of 4-7 years while only one case was below 4 years (1½ year) of age. Two cases were 8 years old. Eleven out of 12 cases were aged 5 years and above.

In the control group 2 cases were below 4 years of age (2 years) while 5 cases fell in the 4 - 7 years range. Remaining 2 cases were 8 years old.

2. Sex distribution

Sex distribution of patients and control cases showed that 9 out of 12 cases of cerebral malaria (75%) were male while only 4 out of 9 (45%) control cases were male (Table-II)

Table - II

Clinical group	Male	Female	M/F ratio	Total
Cerebral malaria	9	3	(3:1)	12
Control	4	5	(4:5)	9
Total	13	8	(13:8)	21

3. Mean Body Weight (Table - III)

Mean body weight expressed as the percentage of Harvard standard median in patients and control cases was quite similar. Among the cases of cerebral malaria only two children had malnutrition of grade II (Indian Academy of Paediatrics).

Table - III

Clinical group	Number	Mean of percentage of weight
Cerebral malaria	12	86.8
Control	9	92.0

4. Causative agent of cerebral Malaria

Out of a total of 12 cases, 7 had evidence of asexual forms of Plasmodium falciparum in their peripheral blood smear. One patient had evidence of asexual forms of both

Table - IV

Haemoglobin, Blood counts and E.S.R. in different clinical groups

Sl. No.	Clinical group	n	Haemoglobin (gm%) Mean (SD)	Total leukocyte count (/cu mm) Mean (SD)	Differential Leukocyte count (%) Mean (SD) P	L	E	M	ESR by wintrobe (mm) in 1st hour Mean (SD)
1.	Cerebral malaria								
(a)	P.falciparum	7	10.06 (1.98)	7828.6 (2930.6)	58.3 (6.5)	38.6 (5.5)	1.7 (1.5)	1.7 (1.3)	36.42 (10.35)
(b)	Mixed infection	1	12.8	9650	41	58	0	1	50
(c)	P. vivax	4	11.30 (0.52)	9300.0 (1925.2)	61.25 (4.97)	35.5 (5.54)	1.0 (1.0)	2.2 (1.1)	40.25 (9.8)
(d)	Total	12	10.70* (1.76)	8470.8 (2613.6)	57.8 (7.8)	32.0 (7.9)	1.3 (1.3)	1.6 (1.2)	38.83 (10.44)
2.	Control	9	12.71 (0.69)	5938.9 (540.5)	59.9 (5.3)	37.5 (5.3)	1.3 (1.1)	1.2 (0.9)	5.11 (4.56)

*The difference from control was statistically significant ($P < 0.05$)

Plasmodium vivax and P. falciparum in the peripheral smear while four patients showed evidence of asexual forms of plasmodium vivax alone.

5. Haemoglobin, Total and Differential leukocyte counts and E.S.R. in different clinical groups

Mean haemoglobin levels, blood count and E.S.R. values of the cases are given in table - IV.

Mean haemoglobin level was significantly lower ($P < 0.01$) in patients than in the control group. However, no statistically significant difference was observed between the mean haemoglobin levels of patients with falciparum infection and with vivax infection. Two patients of falciparum cerebral malaria had haemoglobin levels in the range of severe anaemia (7.2 - 7.4 gm%). None of the patients of vivax cerebral malaria had such low values. Total leukocyte counts showed the mean values within the normal range (4000-11,000/cu mm) in patients as well as healthy control subjects. Differential leukocyte counts also showed normal values in two groups of cases.

E.S.R. values, as measured by Wintrobe's method, were consistently raised in all the cases of cerebral malaria. Mean values were significantly higher in cerebral malaria than in the control group.

6. Duration of fever, Haemoglobin level, Icterus and outcome

As shown in table-V, longer duration of fever was

associated with higher mortality in falciparum cerebral malaria. One patient who had fever for 11 days died while only 1 out of the remaining 6 (17%) who had fever for 3 days or less died. In Plasmodium vivax infection a reverse trend was observed. One patient with longer duration of fever (4 days) survived while 2 out of 3 patients (66%) having fever for 2 days or less expired.

In general, mortality rate was higher in those infected with P. vivax. Two out of 4 cases who had P. vivax infection died. In P. falciparum only 2 out of 7 cases (28.5%) died. However an overall mortality rate was 41.6% (5/12).

There was no significant correlation between the duration of fever and haemoglobin level, in patients infected by P. vivax. The level of haemoglobin was significantly lower with longer duration of fever in the patients infected by P. falciparum. Two patients with P. falciparum infection had haemoglobin levels of 7.2 and 7.4 gm%. Duration of fever in these cases was 11 days and 3 days, respectively. These patients also had clinical jaundice. The sole patient with deeper jaundice and (bilirubin 6.0 mg%) and haemoglobin (7.2 gm%) value died.

Table - V

Species of parasite	Duration of fever	No.	Haemo-globin (mg/dl)	Ieterus	Outcome survi-val	Dea-th
P.falciparum	11 days	1	7.2	+ + +	-	1
P.falciparum	3 days	1	7.4	+	1	-
P.falciparum	< 1 day	5	9.16 (Mean)	(-)	4	1
P. vivax	<< 2 days	3	11.4 (Mean)	(-)	1	2
P. vivax	4 days	1	11.0	(-)	1	-
Mixed infection	No fever at admi-ssion	1	12.8	(-)	-	1
Total					7	5

7. Clinical features

Clinical features of patients of cerebral malaria have been given in the table - VI.

Fever was present in all except one case at the time of development of unconsciousness. The only patient who did not have fever at the time of presentation developed fever during hospitalization. This patient did have fever 15 days prior to admission for which she received chloroquin (oral).

Chills and rigors were present in all the cases having P. vivax infection or mixed infection. While 3 out of 7 patients (42.9%) of P. falciparum infection did not have any definite history of chills and rigors.

Table - VI

Clinical features	P.falci-parum infection	P.vivax infection	Mixed infection	Total
1. Fever				
With chills & rigors	4	4	1	9
Withour chills and rigors	3	-	-	3
2. Pallor				
No pallor	2	2	1	5
Mild	3	2	-	5
Mod./Severe	2	-	-	2
3. Headache	4	2	-	6
4. Vomiting	1	1	1	3
5. Icterus	2	-	-	2
6. Deep coma	7	4	1	12
7. Convulsions				
Generalised	6	4	1	11
Focal	1	-	-	1
8. Focal neurological deficit. Lt.hemip-aresis	1	-	-	1
Lt. facial paresis	1	-	-	1
9. Neuropsychiatric manifestations (confusional state and delirium)	1	-	-	1
10. Spleen (size)				
just palpable on deep palpation	1	-	1	2
0.5 - 1 cm	3	3	-	6
1 - 3 cm	3	-	-	3
7 3 cm	-	1	-	1
11. Liver (size)				
not palpable	1	4	-	5
0.5 - 1 cm	5	-	-	5
7 1 cm	1	-	1	2
12. Loose motions	-	2	-	2
13. Haemoglobinuria	1	-	-	1
14. Hypotension	2	-	-	2

Only two patients infected with P. vivax had mild pallor and the rest were normal. Three patients had mild pallor and 2 had moderate to severe pallor in the group infected by P. falciparum.

Icterus was present in 2 cases of P. falciparum infection. History of headache was present in 4 cases of falciparum malaria and in 2 cases of vivax malaria. History of vomiting (non projectile) was present in one case each of falciparum, vivax and mixed infection.

Deep coma was present in all the cases of cerebral malaria. Convulsions were of generalized type in 11 cases while one case of falciparum cerebral malaria had focal convulsions who later developed focal neurological deficit (left hemiparesis). Hemiparesis persisted during the period of hospitalization (10 days). Initially the patient also had left facial paresis but it disappeared in 3-4 days time.

One patient remained in confusional and delirious state for 3 days following recovery from deep coma.

History of loose motions was present in two cases of vivax cerebral malaria. History of Haemoglobinuria was present in one case.

Hypotension and peripheral circulatory failure was present in two patients of falciparum cerebral malaria who required dopamine drip.

Table - VII

Patient No.	Parasite species	Duration* of coma	C3 in serum (mg/dl)	C3 in CIC (mg/dl)	% C3 in CIC	C4 in serum (mg/dl)	C4 in CIC (mg/dl)	% C4 in CIC	Outcome
1.	P.falciparum	15½ hours	66.0	26.5	40.15	15.25	9.5	62.8	Survived
2.	P.falciparum	1 day	38.75	-	-	10.75	4.25	39.44	Survived
3.	P.falciparum	5 days	17.0	-	-	-	-	-	Expired
4.	P.falciparum	9 hours	59.0	14.0	23.73	16.75	8.0	47.6	Survived
5.	P.falciparum	22 hours	49.0	12.25	25.0	11.0	6.0	54.54	Survived
6.	P.falciparum	11 hours	44.0	8.5	19.32	12.25	7.0	57.14	Survived
7.	P.falciparum	7 hours	74.5	22.0	29.53	34.75	16.0	46.04	Expired
8.	P.vivax + P. falciparum	10 hours	98.5	7.0	7.16	21.25	1.5	7.06	Expired
9.	P. vivax	11½ hours	121.0	17.0	14.23	14.75	10.75	72.88	Expired
10.	P. vivax	7 hours	180.0	12.5	6.94	35.25	22.0	62.41	Survived
11.	P. vivax	13 hours	49.0	7.0	14.29	14.25	7.0	49.12	Expired
12.	P. vivax	8 hours	45.5	22.0	48.35	11.0	7.0	63.63	Survived
n = 12		Mean±S.D.	70.1 ±42.5	12.4 ±8.1	19.1 ±14.5	16.4 ±9.6	8.25 ±5.75	46.8 ±21.3	
		(P value)	(P<0.01)	(<0.2)	(<0.95)	(<0.2)	(<0.3	(<0.01)	
Normal controls	n=9	Mean±S.D.	96.6 ±18.9	17.6 ±7.3	18.7 ±6.9	23.1 ±9.1	5.8 ±3.6	23.8 ±9.6	

*Duration of coma was taken at the time of collection of blood sample.

Status of splenic and liver enlargement, if any, is shown in the table - VI.

Complement (C3 and C4) levels in the serum and circulating immune complexes (CIC's) and their relation to species of parasitic infection, duration of coma and outcome

As shown in the table-VII and VIII C3 levels were significantly low ($P < 0.01$) in the serum of patients of cerebral malaria as compared to normal controls. Two patients had higher than control values of C3 in serum.

Patients with P. falciparum malaria had lower mean values of C3 than those observed in vivax cerebral malaria ($P = 0.05$).

No significant difference was observed in the values of C3 in circulating immune complexes in the patient and control groups. Similarly the percentage binding of C3 to immune complexes as determined by the formula :

$$\% \text{ C3 in CIC} = \frac{\text{C3 in CIC}}{\text{C3 in serum}} \times 100$$

did not show any significant difference in patients and co control cases.

Mean serum C4 value of patients was lower i.e. 75.3% of the normal controls. But the difference was not statistically significant.

Mean serum values of C4 in falciparum malaria were 76% of the values observed in vivax cerebral malaria. Again the difference was not statistically significant.

Table - VIII

Comparision of mean values of complement

Species of parasite	n	Serum C3 (mg/dl)	C3 in CIC (mg/dl)	% C3 in CIC	Serum C4 (mg/dl)	C4 in CIC (mg/dl)	% C4 in CIC
P. falciparum	7	49.7±17.7	11.9±9.4	19.7±13.8	14.4±9.7	7.3±4.6	43.9±19.2
Mixed infection	1	98.5	7.0	7.2	21.2	1.5	7.1
P. vivax	4	98.9±55.7*	14.6±5.5	20.9±16.1	18.8±16.1	11.7±6.1	62.0±8.5

*The difference from P. falciparum was statistically significant P = 0.05

Similarly no significant difference was observed in the values of C4 in circulating immune complexes when patients and control cases were compared. But, the percentage of C4 linked to circulating immune complexes was higher in patients as compared to control subjects and the results were statistically significant ($P < 0.01$). No significant difference was observed between the two species of malaria parasite when their C4 values bound to circulating immune complexes were compared.

There was no correlation between the duration of coma and the level of complement present in the serum or that which was linked to circulating immune complexes.

Duration of coma was also not linked to the outcome of the disease. Also the outcome of disease was unrelated to initial level of complement.

DISCUSSION

DISCUSSION

The present study comprised of 21 children including 12 cases having cerebral malaria and 9 children who were healthy (control). Cases of cerebral malaria were selected strictly according to the criteria laid down by Warell et al (1982). All the patients were in an unarousable coma. All had asexual forms of malaria parasite in their peripheral smears. None of them had any clinical evidence of other encephalopathies.

Control cases were picked up from normal children of hospital staff, or sibs accompanying the patients seen in the O.P.D. or who were admitted in the hospital. Carefully taken history and examination of the control cases excluded any possibility of primary or secondary immunological disorder being present in them. History of any drug intake that would have affected the immune status was also sought to exclude such cases.

1. Age distribution

Age distribution of the patients included in the study was strikingly different from that reported in foreign literature. Eleven out of 12 cases of cerebral malaria were aged 5 years or more. One case was 1½ years old.

Hendrickse et al (1970) found preponderance of cerebral complications mainly under 5 years of age. 144 out of total 171 children who had convulsions were

less than 5 years of age (84.2%) in the Nigerian population studied by them.

Osuntokun (1983), and Bruce Chwatt (1978) and Expert Committee of the W.H.O. Malaria Action Programme (1986) also reported higher incidence of cerebral malaria under 5 years of age in children residing in endemic areas, owing to lower immunity in them. But, on the other hand, Indian literature shows higher incidence of cerebral malaria above 5 years of age as shown in table - IX.

Table - IX

Sl. No.	Name of worker		Reported age group distribution		
1.	Ahmad et al (Aligarh)	1986	0-3 Yrs.(5)	4-7 Yrs.(9)	7-8Yrs.(16)
2.	Sachdeva et al (Delhi)	1985	4-8 Yrs.(3)	8-12 Yrs.(3)	
3.	Kidwai et al (Aligarh)	1986	∠ 2 Yrs.(1)	2-6 Yrs.(5)	6-12Yrs.(13)

Thus, in the present study age incidence was similar to what has been observed by other Indian workers. This finding is against the theory that relative non immunity of children below 5 years of age renders them more susceptible to complications of malaria. It could not be expected that children below 5 years of age residing in Bundelkhand region would remain nonimmune to malaria. In fact, malaria is commonly seen in all the age groups in this region including children below five years of age.

2. Sex distribution

The sex distribution of the patients included in the present study showed male preponderance (75%) (Table-II). This male preponderance was similar to what has been reported by other Indian authors. Sachdeva et al (1985) and Ahmad et al (1986) reported a male female ratio of 2:1. Kidwai et at (1986) reported a ratio of 4:1. Other Indian studies quoted by Ahmad et al viz. Gautam et al (1980) and Patwari et al (1978) also had a male preponderance. Hendrickse et al (1971), Petchclai et al (1977), Adams et al (1981), Srikachul et al (1975) also found similar male proponderance in their series of cases.

3. Mean body weight

On the whole mean body weight of the patients included in the present study did not fall in the category of protein energy malnutrition as per the criteria laid down by the Indian Academy of Paediatrics (table-XII). However, 2 individuals among the group of patients were malnourished (grade II malnutrition). Thus in all about 17% (2/12) cases were malnourished.

It has been observed by Edington (1967) and Hendrickse et al (1971) that convulsions and cerebral malaria were common in well nourished children compared to malnourished cases. Osuntokun (1983) explained this observation on the basis of impaired delayed hypersensitivity and cellular immunity in PEM and associated thymic

atrophy. Relatively low incidence of cerebral malaria in malnourished children in the present study strengthens the previous observations and calls for the need to explain the pathogenesis of cerebral malaria on the basis of immunological mechanisms. Mean body weight (expressed as percentage of Harvard standards) of patients and control cases was almost similar as is evident from table-III. This helped to minimise the variation in complement levels in two groups of cases that would have occured as a result of malnutrition.

4. Causative agent of cerebral malaria

In the present study 4 out of 12 cases showed evidence of asexual forms of P. vivax alone in their peripheral blood smear. A thorough search made in several slides of each patient did not show any evidence of Plasmodium falciparum. Only one out of 12 cases had mixed infection of P. vivax and P. falciparum. Remaining 7 cases were caused by P. falciparum.

It is evident from the above observations that cases of cerebral malaria do occur in Plasmodium vivax infection. All the four cases reported in the present study had the clinical picture resembling classical picture of falciparum cerebral malaria (Table-VI). All the four patients had fever, convulsion(s) and deep coma. The mortality rate was significantly higher than observed in falciparum malaria. Two cases died (50%), while only 2 out of 7

cases of falciparum cerebral malaria (28.5%) died. These mortality figures are similar to that observed by Sachdeva et al (1985) who reported 66% mortality in their series of vivax cerebral malaria.

Rossle (1921) first described a case of vivax cerebral malaria. Taresev et al (1943), Nikolaev (1948), Osiovsky (1949) described cases of cerebral malaria in Russian literature. Jiang et al (1965) reported vivax s cerebral malaria in China. Bruce Chwatt (1978) and Osuntokun (1983) kept only a rare possibility of vivax cerebral malaria. An Expert Committee of W.H.O. Malaria Action Programme (1986) was also, of the same view. Indian literature has several similar reports. Gopinathan et al (1982), Kidwai and associates (1986), Ahmad et al (1986) Varma and Nagotra (1976), Sachdeva et al (1985) all reported cases of vivax cerebral malaria. However, there is a considerable controversy regarding this issue. Kitchen (1949) believed that such cases could be due to an unfavourable premorbid background or intercurrent infection. In the present series all the cases were normal prior to the ailment and two cases did respond to chloroquin therapy alone within 2 days of therapy. If viral encephalites is thought to be an associated factor (Loban and Polozak, 1985) then the present observations go against this since in viral infections an early recovery is quite unusual.

Hill et al (1963), Chabbasse et al (1981) and Loban and Polozak (1985) also kept in the possibility of mixed infection due to P. falciparum infection. But a thorough search in the Giemsa stained smears in the present study, did not reveal any stage of P. falciparum in such cases.

5. Haemoglobin, Total and Differential Leukocyte Counts and E.S.R. (Table-IV)

Mean value of haemoglobin was significantly low (10.7±1.76 gm%) in the patients of cerebral malaria than in the control group (12.7±0.69 gm%) i.e. $P < 0.005$. This findings was similar to the finding of W.H.O. Malaria Action Programme Expert Committee (1986). However, no significant difference was observed when comparison was made between the cases of falciparum (10.06±1.98 gm%) and vivax cerebral malaria (11.3±0.52 gm%). Values of haemoglobin were in the range of severe anaemia (7.2 and 7.4 g%) in two patients of falciparum cerebral malaria according to the criteria laid by the W.H.O. (1986) i.e. haemoglobin level $\leq$ 7.1 gm/dl.

Higher degree of anaemia in falciparum malaria is explained by heavy parasitemia observed in such cases. P. falciparum attacks both early and late forms of RBC's (Chatterjee, 1980). Autoimmune mechanism has been proposed by Rosenburg et al (1973) and Stanley et al (1984). But Greenwood et al (1978)attributed the haemolytic anaemia, observed in P. falciparum malaria, to a higher

degree of invasion of RBC's by the parasite. They could not find any significant autoimmune mechanism operating to explain haemolytic anaemia.

Total leukocyte count in patients of cerebral malaria showed mean value within the normal range (4000-11,000/mm^3) and was not significantly different from the control group. Differential counts were also in the normal range (P 50-75, L 25-50, Eo-4, M 0-8). This was similar to what observed by Schwatz et al (1950), Fisher et al (1970), Reiley and Barret (1971).

E.S.R. was significantly raised in patients of cerebral malaria (38.83±10.44 mm). It was also in conformity with the previous findings (Loban and Poloszak, 1985).

Duration of fever, haemoglobin level, icterus and outcome (Table-V)

Haemoglobin level was low where the duration of fever was prolonged.

No definite pattern was observed between the duration of fever and outcome of illness.

One patient of jaundice died while the other survived.

Vivax cerebral malaria showed higher mortality (50%). One patient who had mixed infection also died. Mortality figure in falciparum malaria was 28.5%.

Clinical features

As is evident from table-VI, fever was present in all the cases of cerebral malaria. One patient had fever 15 days prior to hospitalization for which she received chloroquine and responded to it. She was comatose but did not have fever at the time of admission. However she developed it after one day after hospitalization. The same patient had mixedinfection of P. falciparum and P. vivax. This observation is against the fact that in children fever developed even then relatively mild infection (W.H.O. 1986). On the other hand, expert committee of W.H.O. Malaria Action Programme (1986) states that occasionally the temperature is normal or even subnormal in severe malaria.

Classical chills and rigors were present in all the cases infected by P. vivax. But, three out of seven patients of falciparum malaria did not have a definite evidence of chills and rigors. This observation confirms the view of earlier workers (Bruce Chwatt, 1978, Loban & Polozak, 1985, W.H.O. 1986), that classical picture of malarial fever is generally less prominent in falciparum infection.

Headache was present in 6 out of 12 cases. The headache might have been due to malaria fever itself and not necessarily due to increased intracranial tension. Rise of intracranial tension is not prominent in cerebral malaria (W.H.O. Malaria Action Programme, 1986).

Three patients had vomiting and two other patients had loose motions. Vomiting in these cases was not projectile and was not associated with any drug intake (chloroquin). Thus, in children vomiting could be present in malaria which is some times a prominent complaint (Bruce Chwatt, 1978). This observation calls for a high index of suscicion for malaria in endemic areas.

Pallor was not significant in P. vivax infection and also in the majority cases of P. falciparum infection. Two patients of cerebral malaria who had moderate to severe pallor also had icterus suggesting haemolytic nature of anaemia. In one of the patients deep icterus was not associated with any liver dysfunction as the investigations revealed. This confirms the view expressed by an Expert Committee of W.H.O. Malaria Action Programme (1986). On the other hand this finding is contrary to reports of Ramchandran and Perera (1976) and Martella et al (1969) who found hepatic dysfunction in malaria. Gopinathan et al (1982) have described a malarial hepatitis in nine patients of severe falciparum malaria on the basis of conjugated bilirubinemia and slightly raised alkaline phosphatase. In one of the patients of this series, unconjugated bilirubinemia was present and alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase were normal. Level of bilirubin was 6.0 mg/dl. The other patient with icterus could not be investigated for liver

dysfunction since he was diagnosed as a case of cerebral malaria and responded to antimalarial therapy, promptly.

Convulsions were present in all the cases of present study (100%). This incidence is higher than what has been reported previously by Daraff et al, 1967 (10%), Gopinathan et al, 1982 (15%), Osuntokum, 1983 (50%) and Ahmad et al, 1986 (76.7%). Findings in the present study are quite close to the observations of Sachdeva et al, 1985 (83.3%). This discrepancy can be explained on the basis of strict sampling (according to criteria of Warrel et al, 1986) procedure that has been followed in the present study which made it obligatory to exclude cases of mild impairement of consciousness. It is possible that in mild cases the incidence of convulsion is less. Criteria followed by Sachdeva et al who observed a higher incidence of convulsions (83.3%), were the same as given by Warell et al.

Convulsions were generalized in all but one case who later on was seen to have a left sided hemiparesis when examined after he had recovered his consciousness. Initially for 3-4 days this patient also had left facial paresis which improved later on. But, the pareses of upper and lower limbs persisted even 8 days after the recovery of consciousness. This, confirms the findings of Bruce Chwatt (1978) that sequalae may be present in severe form of cerebral malaria. Marsden et al (1975) also described permanent sequalae in cerebral malaria.

Neuropsychiatric manifestations during the phase of recovery have been described by (Daroff et al (1967), Gopinathan et al (1982) and Osuntokun et al (1983). In the present study there was one patient who had confusion delirium and abnormal behaviour 2-3 days before starting the quinine therapy. He recovered fully confirming the observations made by above mentioned authors. Haemoglobinuria was present in one patient who had deep icterus. This lends evidence for massive haemolysis in that patient who also had severe anaemia (Hb 7.2 gm%).

Spleen was palpable in all the cases. Most of the cases had palpable spleen 0.5 to 1.0 cm below the left subcostal margin. However, splenomegaly was absent in 2 33.3% cases described by Sachdeva et al (1985) and 23.3% cases described by Ahmad et al (1986). Leban and Polcnak (1985) maintained the view that splenomegaly became evident earlier in the patients having previous attacks of malaria. Thus it can be inferred from observations of the present study that children included probably had malaria in the past. Indeed, past history of malaria like illness was recorded in 9 out of 12 cases. This observation is significant since it goes contrary to the theory that cerebral malaria is common in non-immune children (Bruce Chwatt, 1978 and Experiment Committee of W.H.O. Malaria Action Programme, 1986 and Osuntokun, 1983).

Hepatomegaly was present in only 2 patients (7 1cm). Others had either less than 1 cm liver enlargement or else the liver was not palpable. Hepatomegaly is early in younger children (Bruce Chwatt 1978; W.H.O. 1986). In the present study also the only child who was less than 5 years of age (1½ years) had liver size of 3.0 cm, with just palpable spleen.

Signs of collapse and peripheral circulatory failure were present in 2 patients of cerebral malaria. Going by the criteria of Bruce Chwatt (1978), Gopinathan et al (1982), Loban and Polozak (1985), WHO Malaria Action Programme (1986) that multiple complications of malaria could be seen in single patient, picture of algid malaria (collapse) was present in two cases of this study. Indeed, they required dopamine drip besides low molecular weight dextrans for the maintenance of blood pressure.

None of the patients showed any evidence of bleeding. Bleeding and clotting disturbances were previously described as being important pathogenic mechanism in cerebral malaria (Devakul et al 1966), Jaroonvesamma, 1972, Srikamchul et al 1975). But Philips et al (1986) found significant bleeding in only about 5% of patients of cerebral malaria. Present observation is in quite agreement with the finding of Phillips et al (1986). In fact Expert Committee of W.H.O. Malaria Action Programme (1986) observed that disseminated intravascular coagulation leading to

bleeding in cerebral malaria is not an important mechanism for its pathogenesis.

8. Serum complement

As shown in table-VII & VIII mean serum complement C3 level was significantly lower ($P < 0.01$) in patients of cerebral malaria as compared to normal controls.

Mean value of serum complement C4 was low in patients of cerebral malaria being 75.3% of mean values of normal controls. However, the difference was not statistically significant. Mean value of C3 in falciparum was also significantly lower than that found in P. vivax infection ($P = 0.05$).

Mean value of C4 in P. falciparum infection was 76% of mean of observed in P. vivax infection. But the difference was not statistically significant ($P < 0.5$).

Evidence of hypocomplementemia has been found in both experimental and human studies of malaria with or without associated complications. Among the experimental studies, Fegel (1966) and Cooper and Fegel (1966) found depressed levels of complement components C1, C2 and C3 in P. knowlesi infected Rhesus monkeys.

Wright (1968) and Wright et al (1971) noted depressed complement levels in hamsters with P. berghei cerebral malaria.

Houba and Williams (1974) noticed initial increase of C4, C3 associated with peak parasitemia followed by a

decrease of these components far below to their normal range in owl monkeys infected with P. falciparum or P. brasilianum. The scientific group of W.H.O. (1975) reported unpublished data of Krettli et al who found initial increase in the first 3 days of infection followed by a marked fall after fourth day in mice infected with P. berghei.

June et al (1979) observed that serum C3 level in P. berghei infected mice was within the normal range until 9th day of infection when sudden decrease was observed in untreated animals the level continued to fall even after the 9th day. Similar findings of depression of C3 in P. berghei infected mice were observed by contreras et al (1980). Finley et al (1982) also noted depression of C3 in P. berghei infected mice.

Among the human studies since the first report of depression of complement in human malaria by cathoire (1910) and Vincent (1910) there are many similar reports. Dulaney (1948) reported depression of complement in human malaria.

Bhamarapravati et al (1973) noticed depression of C3 and C4 and suggested activation of classical pathway in falciparum malaria.

Rosenburg et al (1973) noted a fall of C3 in falciparum malaria with anaemia.

Greenwood and Brueton (1974) found significantly

depressed values of C3 and C4 in cerebral malaria as compared to normal controls.

Sribaichul et al (1975) found marked depression of C3 in complicated malaria Petchclai et al (1977) also noted a fall in C3 and C4 values besides a similar depression in the values of other complement components. They found evidence of activation of alternative pathway in 2 patients in addition to the activation of classical pathway.

Williams (1978) also reported hypocomplementemia in children with falciparum malaria.

Ahmad et al (1981) reported depressed C3 and C4 levels in cerebral malaria. Gupta et al (1982) reported decrease in C3 level. Phanuphak (1985) noted decrease in C3, C4 and other components with evidence of activation of classical pathway alone. Kidwai et al (1985), Sachdeva et al (1985), Kidwai et al (1986), Ahmad et al (1986) also reported depression of C3 and C4.

Significantly lower mean value C3 in patients with falciparum cerebral malaria as compared to vivax cerebral malaria is in agreement with the findings of Kidwai et al (1985). However,.they did not notice any significant difference between mean C4 values of two species. Similarly in the present study, C4 values in two groups of patients infected with P. falciparum and P. vivax species did not show any significant difference.

Two patients of vivax cerebral malaria had higher values of C3 than the mean value seen in healthy controls. Two other patients, one with vivax cerebral malaria and another with falciparum cerebral malaria had significantly higher mean C4 value than that seen in normal controls.

These deviations from general trend can be explained on the basis of, acute phase response to infection where increased production may mask the utilization (Petchelai et al, 1977 and Phanuphak et al 1985). Similar findings have been observed by Lambert and Mouba in experimental models, where the levels of C3 and C4 increased during the peak of parasitemia followed by a fall. Jume et al (1979) also found normal values of C3 and C4 like Kretti et al, in the initial phase of infection followed by a fall, in their experimental models.

Since both C3 and C4 levels are depressed it can be reasonably inferred that activation of classical complement pathway occurred in cases of cerebral malaria in the present study.

Present observation regarding C3 values being significantly lower in P. falciparum than P. vivax group in the presence of almost equal C4 values, is difficult to explain. However, petchelai et al (1977) and Stanley et al (1984) noted activation of alternative pathway in falciparum malaria besides classical pathway. Stanley (1984) found that surface erythrocytes infected by trophozoite stage

of P. falciparum activated alternative pathway. Thus, activation of alternative pathway, resulting in increased consumption of C3 in falciparum malaria could presumably explain the present finding. No comparable report could be traced in the literature showing activation of alternative pathway in vivax malaria. On the other hand, Phanuphak et al (1985) and Greenwood and Brueton (1974) did not find any evidence of activation of alternative pathway in falciparum malaria.

There was no correlation between levels of C3 and C4 in the serum and duration of coma. Similar observations were made by Greenwood and Bruton (1974).

There was also no correlation between the outcome of disease and the value of complement C3 and C4 in the serum. Out of the two patients, both of whom had very low levels of C3, one did not have any detectable level of C4 as well. The other patient had very low level of C4. First of these two patients died while the other one survived. But the patient who survived did not initially respond to chloroquin and grade I unconsciousness persisted till quinine therapy was started. However, in general, levels of C3 and C4 did not correlate with the outcome. This finding is in variance with Ahmad et al (1986) and Kidwai et al (1986) who found positive correlation between complement values (C3 and C4) and the survival. Petchalai et al (1977) and Srikajchul et al (1975) also found

relation between hypocomplementemia and complications in falciparum malaria. Greenwood and Brueton (1974), Phanuphak et al (1985) and Fribourg et al (1986) did not find any correlation between the levels of complement in two groups of the patients i.e. malaria and cerebral malaria.

These authors have concluded that since there was no significant difference of complement levels between these two groups of malaria cases it could be reasonably inferred that there was no significant difference of complement levels between better prognosis and poor prognosis. However these authors did not give data of outcome in their patients of cerebral malaria.

C3 and C4 in circulating immune complexes (CIC)

Polyethylene glycol (PEG) precipitation method is a non specific method of separation of immune complexes. Various authors like Chia et al (1977) and Jana et al (1982) have found that material precipitated by PEG at low concentrations consists of immune complexes.

In the present study 4% polyethylene glycol (PEG) was used to precipitate immune complexes. Redissolved precipitate (CIC) obtained from serum of each case was subjected to complement study.

As shown in the table-VII and VIII neither the level of C3 in circulating immune complexes nor percentage of C3 bound to circulating immune complexes was significantly raised in patients, in comparison to healthy controls.

However, mean percentage of C4 linked to circulating immune complexes was significantly higher ($P < 0.01$) in patients than that in controls.

In the patients of systemic lupus erythematosus significantly higher percentage of C3 in circulating immune complexes is due to its linkage to small immune complexes (Chia et al 1977). Since there was no significant difference in the percentage C3 linked to CIC in patients and control cases, in the present study, one can assume that small complexes were not in higher amount in patients of cerebral malaria as compared to controls. On the other hand, higher percentage of C4 linked to CIC in patients than in controls points out the presence of those complexes in the sera of the patients which activate classical pathway. Complement C4 is a part of classical pathway. According to Haynes and Fauci (1987), immune complexes of Ig M-Ig G class activate classical pathway of complement system while immune complexes of Ig A class activate alternative pathway. Thus itcan be said that in present study immune complexes of Ig G - Ig M class were, probably, present in a significant amount in the patients and were probably of moderate to large size. This is not a new observation. A number of workers have reported formation of immune complexes in experimental as well as human malaria with or without complication which has been associated with the pathogenesis of complications of malaria.

Formation of immune complexes, their deposition in glomerular basement membrane leading to nephrotic syndrome in Plasmodium malariae infection is proved beyond doubt (Ward et al, 1969, Allison et al, 1969, Idris Mohd. 1982). Certain authors like Dhamarapravati et al (1973) have also found evidence of soluble immune complexes and their deposition in glomerular basement membrane in the patients of falciparum malaria presenting with glomerulonephritis. Houba et al (1979) also proposed immune complex mechanism in falciparum malaria presenting as glomerulonephritis.

Similarly, immune complexes have been reported by Weis (1978), Boonpucknavig et al (1979), June et al (1979), Contreras et al (1980), Finley et al (1982) in mice infected by P. berghei. Ehlrich et al (1981) noticed elevated circulating immune complexes and their subsequent deposition in kidneys of P. falciparum of P. berghei infected rats.

June et al (1979) found evidence of deposition of immune complexes in the choroid plexus of mice infected with P. berghei and having cerebral malaria. Observations of Finley et al (1982), were no less important. They noticed that intact immune system is necessary for the expression of cerebral malaria. In their experimental models of T. cell dependent mice and T. cell independent mice, cerebral malaria was more severe in T-cell independent mice, where immune system was intact. Formation of circulating immune complexes was also more in such mice.

Findings, of Finley et al support the previous findings of Wright (1968) and Wright et al (1971) who found that in neonatal thymectomy or administration of antithymocyte serum in golden hamsters infected by P. berghei almost suppressed the development of acute haemorrhages of the brain due to an intravascular antigen-antibody reaction.

Thus, there is enough experimental evidence to support immune complex nature of cerebral malaria in animals. All of the above, workers also noticed simultaneous hypocomplementemia with the formation of circulating immune complexes pointing out that complement activation by immune complexes may be an important pathogenic mechanism.

Similarly, among human studies, Perin et al (1979) and Srikaichul et al (1975) found evidence of circulating immune complexes associated with hypocomplementemia in falciparum malaria. Srikachul et al (1975) proposed that disseminated intravascular coagulation and release of kinins secondary to activation of complement by immune complexes, could be considered important mechanism for complications of cerebral malaria.

Previously, Greenwood and Brueton (1974) hypothesized a mechanism quite similar to Srikaichul et al (1975). They also found evidence of immune complexes and their association with hypocomplementemia. Phanuphak et al (1985) also found immune complexes in falciparum malaria with or without complication. But like Srikaichul et al

they could not find any correlation between the level of complement and immune complexes.

Adam et al (1981) showed that circulating immune complexes were found in cerebral malaria, and that their formation was associated with the reduction in complement levels. One of the most significant finding in their study was the deepening of coma in some patients after starting quinine therapy which was associated with the release of antigens of malaria parasite and their interaction with antimalarial antibodies (Ag-Ab complex formation). Toro and Roman (1978) proposed that since cerebral malaria was a disseminated vasculomyelinopathy it could result from a 'hypergic' reaction of CNS to falciparum antigens. They proposed an immune complex vasculitis of brain vasculature.

Sachdeva et al (1985) also reported formation of circulating immune complexes in cerebral malaria.

According to Idris Mohammed (1982) immune complexes could cause cerebral malaria either by their deposition into the vasculature of brain or by causing other reactions while being in circulation itself. Though deposits of immune complexes in brain were found by June et al (1979) in their study over mice, Mcphersan et al (1985) did not find any evidence of immune complex deposits in human brain, on autopsy of patients of cerebral malaria.

Whatever may be the mechanism in the formation of circulating immune complexes, their association with

hypocomplementemia does point to activation of complement system by immune complexes. Complement activation itself may cause damage like, shock and disseminated intravascular coagulation.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present study was conducted in the department of Paediatrics, M.L.B. Medical College, Jhansi, over a period of one year, from Aug. 1987 to Aug. 1988.

Twenty one cases between 1½ years to 8 years of age were selected for the present study. Our sample included 12 patients which were selected on the basis of strict criteria laid down by Warell et al (1982) for the diagnosis of cerebral malaria. Remaining 9 cases were healthy children which served as control for the present study.

A detailed history was taken and a thorough clinical examination was done in all the cases. A complete record of treatment given and progress of the patients, during hospitalisation was kept. Haemoglobin level, Total and Differential Leukocyte count and E.S.R. (by Wintrobe's method) were determined in all the subjects. Serum complement C3 and C4 were quantitated in each case by single radial immunodiffusion. Circulating immune complexes were separated by using 4% polyethylene glycol. Linkage of complement components C3 and C4 to circulating immune complexes was also determined.

The observations and inferences drawn from this study are summarised below :

1. <u>Age distribution</u>

Age distribution of patients was similar to what

has been observed in other Indian studies. Eleven out of twelve cases were aged 5 years or more, contrary to observation of foreign authors that children under 5 years of age were more susceptible to cerebral malaria due to their low immunity to malarial infection.

2. Sex distribution

A male preponderance was observed in the present study which is similar to that observed by other workers in the field.

3. Causative agent of cerebral malaria

Out of twelve patients four patients had P. vivax infection alone, seven had P. falciparum infection and one patient had mixed infection by these two parasites. Our finding of cerebral malaria caused by P. vivax called for the need to reevaluate P. vivax as a possible aetiological agent in the causation of cerebral malaria.

4. Mean body weight

Mean body weight (expressed as percentage of Harvard standards median) were almost similar between patients and controls.

Most of the patients did not fall under the category of protein energy malnutrition as per criteria laid down by Indian Academy of Paediatrics, confirming the observations of previous authors that cerebral malaria is less common in malnutrition. This emphasized the need to explain pathogenesis of cerebral malaria on immunological

basis, since malnutrition is known to depress the immunity of an individual.

5. Haemoglobin, Total and Differential Leukocyte Counts and E.S.R.

Mean haemoglobin level in patients was significantly lower than that observed in controls. E.S.R. was consistently raised in all the patients. Total and Differential Counts were within the normal range in the patients as well as control group of cases.

No significant difference was observed in these parameters when results were compared between patients of falciparum cerebral malaria and vivax cerebral malaria.

6. Duration of fever, haemoglobin level, icterus and outcome

It was observed in the present study that haemoglobin level was low in those cases of cerebral malaria where duration of fever was prolonged.

It was seen that only 2 out of 12 patients studied had significant jaundice and simultaneous very low haemoglobin values, suggesting that perhaps anaemia was because of haemolysis caused by the parasitic invasion of erythrocytes. However, it was seen that low level of haemoglobin was unrelated to the outcome.

No correlation was found between duration of fever and outcome of the illness.

A significant finding in our study like Sachdeva et al (1985) was higher mortality (50%)observed in the case of vivax cerebral malaria as compared to that observed in the cases of falciparum cerebral malaria (28.5%).

The overall mortality observed by us in present study was 41.5%.

7. Clinical features

Clinical profile of the patients studied, in general was similar to previous studies.

Convulsions were present in all the cases unlike other studies. This discrepancy was explained on the basis of strict sampling procedure which made it obligatory to exclude the cases of mild cerebral dysfunction, having, probably, lower incidence of convulsions.

Neuropsychiatric manifestation and focal neurological deficit were observed in one case each.

No evidence of disseminated intravascular coagulation was found in any case.

One comatose patient did not have fever at the time of presentation underlying the need for high index of suspcion for the diagnosis of cerebral malaria.

Yet another patient had deep icterus (serum bilirubin 6.0 mg%) emphasising the need not to get confused with hepatic coma in such a case.

8. Serum complement (C3 and C4) levels

Mean serum C3 level was significantly depressed in association with lower mean level of C4 in patients when compared to corresponding mean values in controls, suggesting activation of classical pathway of complement in cerebral malaria.

Mean serum C3 value was significantly lower in patients with falciparum infection than that with vivax infection in the presence of almost similar mean serum C4 values observed in two types of infections. This observation suggested the probability of activation of alternative pathway as well in falciparum malaria.

No relation between degree of hypocomplementemia was observed either with duration of coma or with prognosis of the patient.

9. Linkage of complement C3 and C4 to circulating immune complexes

Circulating immune complexes were found in all the cases of cerebral malaria. significantly higher percentage of complement C4 was linked to circulating immune complexes as compared to controls.

No significant difference was observed in the percentage of C3 bound to circulating immune complexes between patients and controls.

Complement C3 had been found to be linked with smaller complexes in some studies. It was, therefore,

inferred that immune complexes detected in the patients included in the present study were probably of large size.

Significantly higher binding of C4 to circulating immune complexes in our patients, than the control group of cases, suggested a probability of presence of immune complexes of Ig-G, Ig M class which activate classical pathway of complement system which includes C4.

No significant difference was observed between the mean values of percentage of C3/C4 linked to immune complexes in the two types of malarial infection i.e. falciparum and vivax infection.

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APPENDIX

APPENDIX

CASE RECORD

A STUDY OF COMPLEMENT IN CHILDREN WITH CEREBRAL MALARIA

Department of Paediatrics, M.L.B.Medical College,Jhansi

Case No.	M.R.D. No.	
Patient's name	Age/Sex	
Ward/Bed	D.O.E.	
Address	D.O.A.	
	D.O.D.	
Consultant	Weight	

H/O PRESENT ILLNESS

A. Fever
- Chills and rigors — Yes/No
- Duration
- Type-intermittent — Remittent — Continuous

B. Altered sensorium
- Onset-Sudden/Acute/Gradual
- Duration

C. Convulsions
- Tonic — Clonic — Both — Others
- Onset (in relation to fever)
- Generalised — Focal
- Focal area

D. Vomitings
- Projectile/Non-projectile
- After drug intake/without drug intake (e.g.chloroquin)
- Frequency — Duration

E. Headache — Yes/No

F. Loose motions — Yes/No — Duration
- Frequency — Blood in stools — Yes/No

G. Haemoglobinuria
- Time after fever
- Duration

PAST HISTORY

- H/O malaria (dates if available)
- H/O convulsions (details)
  - With fever/without fever
  - Generalized/focal Tonic/clonic/both

PERSONAL HISTORY

A. Perinatal history
  - H/O birth asphyxia
  - Birth trauma
  - Neonatal jaundice
  - Neonatal infections

B. Developmental history
  - Motor Delayed/normal Manipulative Delayed/Normal
  - Speech Delayed/normal Social Delayed/Normal

CLINICAL EXAMINATION

A. General
  - G.C. Pallor
  - Unconsciousness (grade) Jaundice
  - P.R. Cynosis
  - R.R. Clubbing
  - Temp. Oedema
  - B.P. Dehydration
  - Pupils Significant lymphadenopathy
  - Behaviour

B. Nervous system
  - Cranial nerves
  - Motor system
    - Posture Decerebrate/decorticate
  - Tone - Upper limb - R / L Lower limb - R / L
  - Power - Upper limb - R / L Lower limb - R / L
  - DTR KNEE ANKLE BICEPS TRICEPS
    R
    L

- Supervicial reflexes : Abdominal Cremastic Plantars
  R
  L
- Involuntary movements (including convulsions)
- Sensory system
- Signs of meningeal irritation

C. Abdomen
- Liver Size
- Spleen Size

D. Respiratory system

E. C.V.S. Signs of PCF
Signs of CHF

F. Skin Any evidence of bleeding manifestation
1. From punctured wound
2. G.I. System
3. Purpura/ecchymosis

TREATMENT GIVEN

Date	Treatment given

PROGRESS CHART

Date	Clinical features

INVESTIGATIONS

Blood

A. Peripheral smear
Species of malaria parasite Stage
Signs of haemolysis Yes/No

B. Routine -- Date and time of sample collection
- TLC DLC
- Hb ESR
- PCV

C. Serum -- Date and time of sample collection
1. Complement C_3 mg%
2. Complement C_4 mg%

D. PEG - precipitate (circulating immune complexes)
1. Complement C_3 mg%
2. Complement C_4 mg%

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